Multiple processes in the short-term reduction of palatability in mice

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Time since recent consumption is an important factor in determining eating behaviour, due to the occurrence of short-term adaptation. This adaptation effect is seen in the amount consumed being reduced and is also associated with a corresponding reduction in palatability of the food recently consumed. In this series of experiments the time course of this short-term adaptation effect is investigated, using mean lick cluster size as a measure of palatability during consumption of sucrose solution in mice. It is firstly demonstrated that there is a reduction in the total number of licks as well as the mean lick cluster size after recent consumption of a sucrose solution. This is found to occur rapidly with consumption and also to recover over short time periods between feeding opportunities. However, rather than there being a single short-term adaptation effect, there is found to be an inverted U-shape function of palatability with time since recent consumption. Two experimental confounds that may have resulted in the secondary decline in palatability are subsequently investigated. Firstly a frustrative non-reward account is tested, before differing time in the context before consumption is also investigated. As the secondary decline in palatability remains despite these factors being accounted for, it is concluded that there are two short-term adaptation processes that occur after recent consumption of a sucrose solution.

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Chapter One

Introduction

Understanding eating behaviour and the regulation of energy intake is important for gaining an insight into the conditions in which such regulation is impaired, for example during obesity and anorexia. There are many factors that influence eating behaviour, including previous experience and the hedonic value of the food being consumed. There are also various homeostatic mechanisms that act to regulate eating behaviour (Rolls, 2015; Woods & Langerhans, 2012; Woods & Begg, 2015). One important factor is that of time since consumption, due to the occurrence of short-term adaptation after recent intake of food. This adaptation effect is seen in the form of reduced intake as well as a reduction in the palatability of the food being consumed (Berridge, 1991; Havermans, Janssen, Giesen, Roefs & Jansen, 2009; Rolls, Rolls, Rowe & Sweeney, 1980). This adaptation has been demonstrated in a range of both human and animal studies to be specific to the food recently consumed (Rolls, Duijvenvoorde & Rowe, 1983; Rolls, Duijvenvoorde & Rolls, 1984). It has also been found to occur rapidly after consumption (Hetherington, Rolls & Burley, 1989; Hsiao & Tuntland, 1971). Together this evidence suggests a role for sensory processes in the short-term adaptation that occurs after recent consumption, however the psychological and neural basis of this effect remains unclear.

1.1 Measuring palatability, a microstructural analysis of licking

To investigate palatability in terms of its psychological and neural basis, it is important to be able to objectively measure the construct in animal subjects as well as humans. Grill and Norgren (1978) devised a measure of taste reactivity based on orofacial responses of rodents, observed during the consumption of various solutions infused directly into the mouths of rats. They found that there were characteristically different orofacial responses to sweet tastes such as sucrose than to bitter tastes such as quinine. These consistent responses were categorised into either appetitive (Grill & Norgren termed ingestive) or aversive responses. Sweet sucrose solutions resulted in appetitive taste reactivity responses, such as rhythmic tongue protrusions, whereas bitter quinine produced aversive responses including gaping. Similar orofacial responses have been observed across mammalian species (Berridge, 2000; Steiner, Glaser, Hawilo & Berridge, 2001;) providing evidence for taste reactivity based on these orofacial responses to be a good measure of palatability.

Hedonic responses are not a fixed property determined by the sensory properties of the food being consumed, but can be altered. This has been demonstrated in studies using human ratings of pleasantness, which have been found to vary depending on the physiological state of the subject (e.g. Cabanac, 1971). Taste reactivity measures have also been demonstrated to vary with the physiological need state of the animal, further supporting its use as a measure of the hedonic response to a solution being consumed. For example, the induction of a sodium deprivation alters the normal orofacial responses to salt solutions, from a combination of aversive and appetitive to solely appetitive when deprived (e.g. Berridge, Flynn, Schulkin & Grill, 1984). Not only

are such changes in the hedonic response seen after changes in the physiological state of the animal, but also previous experience and effects of learning can be seen. For example, pairing a liked sweet solution with un-palatable lithium chloride results in an increase in the aversive taste reactivity responses and reduces the appetitive responses normally seen (Breslin, Grill & Spector, 1992; Pelchat, Grill, Rozin & Jacobs, 1983).

These examples in which taste reactivity can be used to measure changes in palatability, provide further evidence that it can be used as a measure of the hedonic response during consumption. There are however, limitations of this method, including the requirement of human coding of the orofacial responses, as well as the responses being categorised into either appetitive or aversive responses only. Furthermore, the orofacial responses are coded using very brief time frames during consumption, allowing only a limited time scale of the taste responses to be analysed. To overcome the problems with taste reactivity another measure of the hedonic response to food has been used, that of the microstructure analysis of licking.

This measure centres on the observation that licking occurs in rapid runs of licks, many of which group together to form lick clusters separated by pauses in licking. Not only can the total number of licks made during consumption be recorded, provided a measure of intake, but the mean lick cluster size can also be recorded (Davis, 1973; Davis & Smith, 1992). Furthermore, the pattern of these measures has been found to vary as a function of sucrose concentration, with the two different measures differentially affected. Whereas lick cluster size shows a monotonic increase with increasing sucrose concentration, consumption follows an inverted U-shape function

(e.g. Austen, Strickland & Sanderson, 2016; Davis & Smith, 1992; Davis, 1996; Dwyer, 2008; Spector, Klump & Kaplan, 1998). Therefore an increase in lick cluster size can occur independently of an increase in the total number of licks. This demonstrates that intake may not be the best measure of the hedonic response to food, as the same amount can be consumed at both high and low solution concentrations with very different lick cluster sizes (e.g. Davis & Smith, 1998; Dwyer, 2008).

The monotonic increase seen in lick cluster size with increasing sucrose concentration, which dissociates from consumption following an inverted U-shape function, suggests that it may be used as a measure of palatability during consumption. Further evidence for this comes from lick cluster size not only increasing as a function of the concentration of a sweet solution (e.g. Davis & Smith, 1992; Davis & Smith, 1998) but also decreasing with increasing concentration of a bitter and unpalatable solution such as quinine (e.g. Hsiao & Fan, 1993; Spector & St John, 1998). The hedonic value of food however, is not only determined by the sensory properties and concentration of the solution but also alters as a result of physiological state and previous experience. For example lick cluster size has been found to reduce with conditioned taste aversions, in which a liked palatable solution, such as saccharin or fructose, is paired with non-palatable Lithium Chloride (e.g. Breslin, Spector & Grill, 1992; Dwyer, Boakes & Hayward, 2008; Dwyer, 2009).

Further evidence that the lick cluster size can be used to measure changes in palatability that occur as a result of previous experience, comes from the negative contrast effect. During this procedure, consuming a low 4% sucrose solution after previous training with a higher 32% concentration, results in a smaller mean lick

cluster size than if previous training with 4% had occurred (Austen & Sanderson, 2016; Grigson, Spector & Norgren, 1993). As well as learning effects such as conditioned taste aversions and negative contrast both manipulating lick cluster size and taste reactivity similarly, pharmacological manipulations also appear to affect both measures. The administration of benzodiazepine for example has been found to increase appetitive responses in taste reactivity paradigms (e.g. Gray & Cooper, 1995; Treit & Berridge, 1990) as well as increase the mean lick cluster size (Higgs & Cooper, 1998), suggesting both measure the hedonic value during consumption.

For lick cluster size to provide a measure of palatability beyond the total number of licks, which necessarily correlates with the volume consumed, the two measures need to be shown to be dissociable. Such a dissociation can be seen in the different functions with increasing solution concentration, meaning that despite consumption being the same at both high and low concentrations the lick cluster size can differ greatly. Further evidence for this dissociation between the two measures can be seen in the manipulations that affect the mean lick cluster size and volume consumed differently (e.g. Dwyer, 2008; Dwyer, Boakes & Hayward, 2008). Overall lick cluster size appears to provide a good measure of the hedonic value of foods during consumption. Therefore any manipulations altering lick cluster size can be concluded to be also altering the hedonic value of the solution, even when the actual concentration of the solution remains the same. Furthermore if the palatability of the solution alters without any change in the solution, this suggests that the perceived sweetness of the solution changes in a way that is analogous to a physical change in solution concentration (Dwyer, 2008; Dwyer, 2012; Harris & Thein, 2005).

This change in the perceived or acquired sweetness of the solution can explain the results found when learning effects alter the palatability despite the solution being consumed not actually changing in sweetness. During conditioned taste aversion procedures (e.g. Dwyer et al 2008; Dwyer, 2009) the liked solution that previously produced large lick clusters during intake, results in both a reduced lick cluster size and consumption. This is the same pattern as would be expected to occur if there was a change in the solution from a sweet palatable to an unpalatable one, such as bitter quinine (e.g. Hsiao & Fan, 1993; Spector & St John, 1998). Similarly the negative contrast effect (e.g. Austen & Sanderson, 2016; Grigson et al, 1993; Dwyer, Lydall & Hayward, 2011) has also been suggested to be due to changes in the sensory nature of the solution, with the solution paired with the higher concentration resulting in a decrease in the perceived sweetness of the solution compared to a group never exposed to the higher concentration.

The effects on lick cluster size during flavour preference conditioning (Dwyer et al, 2008; Harris & Thein, 2005; Sclafani, 2002) can also be explained by an effect that alters the perceived sweetness of the solution. Specifically, the preference for the CS+ flavour when given with a lower concentration at test, can be explained by the flavour paired with a higher concentration becoming sweeter compared to the flavour previously paired with a lower concentration. Furthermore, if there is an increase in the perceived sweetness in a way that is analogous to an actual increase, then it would be expected that consumption and palatability should follow the same inverted U and monotonic functions with increasing perception of sweetness.

Such a result has also been shown using flavour preference conditioning when testing both the CS+ and CS- flavours with different concentrations of sweet solutions (Harris & Thein, 2005; Sclafani, 2002). If the CS+ increases the sweetness in addition to the concentration present at test, then it would be predicted that giving a high concentration paired with the CS+ would not increase consumption if this level of sweetness then goes beyond the peak of the U-shape function. The mean lick cluster size, however, follows a monotonic increase, meaning that it should continue to increase irrespective of the actual concentration presented at test. This lack of preference for the CS+ when it was paired with a high concentration solution during test sessions was found by Harris and Thein (2005), in which test sessions with 5% and 30% sucrose solutions were compared. They found that although there was a preference in the form of increased intake for the CS+ flavour when paired with a 5% solution, there was no such preference when the flavours were presented with 30% sucrose at test. Similar results were also found by Sclafani (2002) in which the flavour paired with 5% sucrose in training was subsequently preferred over one trained with 30% when paired with either 17.5% or 30% at test, whereas this preference was reversed when given with 5% during test sessions.

These results support the idea that the concentration of solution present at test combines with the perception of sweetness retrieved by the solution, in this case a flavour, meaning that in some cases the solution may become perceived as being too sweet. As a result of this, rather than an increase in consumption for the CS+ there may actually be a decrease under some situations compared to the CS- flavour, the pattern of which depends on the perceived concentration in relation to the inverted U-

shape function of consumption. Furthermore, Dwyer (2008) also found that there was a preference for the CS+ in terms of a larger lick cluster size when presented with a low 2% concentration solution as well as a higher 16% one, whereas consumption was only greater for the CS+ with the 2% concentration. Again this supports the idea that there is an effect which increases the sweetness of the solution in a way similar to an actual alteration of concentration, in that there is an increase in consumption only at lower concentrations but that lick cluster size continues to increase with sweetness perception.

Overall palatability can be measured not only through the use of taste reactivity, a measure with various limitations, but also through the lick cluster size during consumption, a measure which is dissociable from consumption and the total number of licks. The use of such a microstructure analysis of licking has shown that changes in palatability and consumption that occur with various manipulations follow the same patterns as would be expected if the solution itself had changed. This suggests that changes in eating behaviour relate to an effect involving a change in the perception of the sweetness of the solution during consumption.

1.2 Short-term adaptation to flavours

One well-documented factor influencing eating behaviour is time since consumption. This is due to short-term adaptation, a change in behaviour as a result of an event, occurring after recent intake. In particular there has been demonstrated to be a decline in acceptance of a food being consumed (e.g. Epstein, Rodefer, Wisniewski & Caggiula, 1992; Swithers, 1996) which occurs until eating of the food ceases. However, this reduction in intake also recovers when a new food is presented, suggesting that the short-term adaptation it is specific to the food being consumed. The finding that food intake declines with consumption as well as meal variety enhancing intake has been widely documented in human studies (e.g. Cabanac, 1971; Hetherington, Rolls & Burley, 1989; Rolls, Rowe, Rolls, Kingston, Megson & Gunary, 1981). For example, one such study by Rolls, Duijvenvoorde and Rolls (1984) found that intake was increased by 60% when a variety of foods were presented compared to when the same course was re-presented for the same number of courses. This reduced consumption of a recently consumed food, as well as food variety being seen to increase intake, has also been found in variety of animal studies including rats (e.g. Rolls, Duijvenvoorde & Rowe, 1983; Young, 1940) and primates (Burton, Rolls & Mora, 1976; Rolls, 1981; Rolls, 1989). Short-term adaptation to food therefore occurs across species and appears specific to the sensory properties of consumed foods.

This short-term adaptation effect appears to correspond with a decline in palatability, a process which is again specific to the food being consumed and may relate to the reduction in consumption. Such a reduction in palatability has been found in a range of human studies and also appears to correlate with the decline in consumption (e.g.

Havermans, Janssen, Gisen, Roefs & Jansen, 2009; Hetherington, Rolls & Burley, 1989; Rolls, Rolls & Sweeney, 1981). Similarly, Berridge (1991) found a decline in the affective taste reactivity responses in rats after pre-feeding with the same milk or sucrose solution, but not when the solution was different. Evidence for this reduction in palatability using mean lick cluster size is limited, however Dwyer (2012) describes how when rats are presented with repeated 60 second exposures to a palatable sweet solution, lick cluster size declines over successive presentations. Short-term adaptation therefore appears to consist of a reduction in consumption parallel to a reduction in the hedonic value of the food being consumed, with some evidence for this process being related to the sensory-specific properties of the food being consumed.

Further evidence for this short-term adaptation being related to a sensory process comes from changes in consumption and palatability occurring after consumption of non-calorific, but palatable, solutions. If the short-term adaptation effect is due to the calorie and nutrient intake then this should not be seen when using such solutions. However, if it relates to just the sensory properties of the food then it will remain. Wooley, Wooley and Dunham (1972), found that in humans consumption of a non-calorific sweetener (Cyclamate) resulted in the same decline in pleasantness ratings during subsequent consumption of a sucrose solution as glucose. As well as this lack of calorie content resulting in short-term adaptation, Rolls and Rolls (1997) also found that chewing (without swallowing) or smelling a food for a duration approximating the time it would be in the mouth during consumption, resulted in a significant reduction in pleasantness ratings compared to foods not consumed. Similar evidence has also been found in rats (Hsiao & Tuntland 1971; Jones, 1970) with consumption of non-

nutritive saccharin or cyclamate subsequently reducing intake of a glucose solution to the same extent as previously consuming a glucose solution

The rapid time course of the reduction in consumption and palatability further supports the sensory properties of the food being important during short-term adaptation. For example in a human study by Hetherington, Rolls and Burley (1989), the time course of the short-term adaptation was investigated using ratings of pleasantness after various consumption intervals. It was found that the greatest decrease in pleasantness occurred just 2-minutes after consumption before gradual recovery over the subsequent hour. The influence of sensory-related mechanisms on consumption should be seen far sooner than any processes relating to negative feedback, due to such mechanisms being reliant on digestion of the food having occurred (Booth, 2001; Cabanac, 1971). Similar evidence from animal studies however is limited, due to the difficulty of using taste reactivity to track palatability over periods of time. Berridge (1991) however, found a reduction in taste reactivity responses only 1-minute after pre feeding, providing some evidence short-term adaptation also has a rapid time course in rats. Overall, short-term adaptation in the form of a reduction in consumption and palatability has been demonstrated in both human and animal studies. Furthermore, this adaptation effect appears to follow a rapid time course suggesting a role for a sensory mechanism as opposed to post-ingestive negative feedback.

1.3 Potential mechanisms of short-term adaptation

There are a number of potential explanations for the decline in consumption and palatability seen during the short-term adaptation that occurs after recent consumption. One explanation is sensory adaptation in which reduced responsiveness to the food being consumed may occur, reducing the palatability and amount consumed as a result. In humans there is some evidence suggesting that the short-term adaptation does not relate to a reduction in the perceived intensity of the food, something that would occur with reduced responsiveness to a food. Rolls, Rolls and Rowe (1983) for example, found that although pleasantness ratings declined with consumption, there were only very minor changes in ratings of intensity. The role of sensory adaptation in the short-term adaptation effect seen after consumption in animal studies however cannot be ruled out.

As well as sensory adaptation, fatigue could also result in a general reduction in responding to food after consumption. The recovery of the adaptation effect when a new food is presented however (e.g. Epstein, Rodefer, Wisniewski & Caggiula, 1992; Rolls, Duijvenvoorde & Rowe, 1982), suggests that a general fatigue mechanism does not result in the reduction in responding seen after consumption. Furthermore, in studies using lick cluster size as a measure of palatability, the mean cluster size continues to increase as a function of solution concentration (e.g. Davis & Smith, 1992; Dwyer, 2008) indicating that responding is able to occur to a much greater extent than seen with intermediate concentrations used to maximise consumption. As well as sensory adaptation and fatigue the decline in responding could also be due to short-term memory processes (e.g. Wagner, 1981). In particular the representation of a

stimulus generated from its presentation may result in habituation of behavioural responding, with this representation decaying quickly from short-term memory for recovery to occur. Although short-term memory may explain the reduction in responding, there is another factor to consider in relation to adaptation that occurs after consumption, that of post-absorptive negative feedback mechanisms.

Despite the immediate rapid decline in consumption and palatability appearing to relate to the sensory properties of the food, eating behaviour is also regulated by the influences of digestive mechanisms, which will have a slower time course than sensory related satiety (Fantino, 1984; Rolls, 1986, 2015; Woods & Begg, 2015). Evidence for the influence of such mechanisms can be seen in satiety studies observing the decline in palatability and consumption over longer periods of time than would be relevant to sensory mechanisms (Cabanac, 1971; Duclaux, Feisthaue & Cabanac, 1973). For example Cabanac (1971) investigated changes in consumption and pleasantness ratings in human subjects after consumption of high concentration glucose solutions, finding that the decline in palatability was gradual and reached a maximum after 45-60 minutes. This slow time course led to the conclusion that post-absorptive effects, such as stimulation of receptors in the duodenum, result in the hedonic changes seen with satiety. As ratings were only gathered from 20-mintues onwards however, any occurrence of a more short-term adaptation effect prior to this decline is unknown.

There are a variety of homeostatic mechanisms influencing intake after digestion including adiposity signals such as insulin and leptin (e.g. Baskin, Latteman, Seely, Woods, Porte & Schwartz, 1999) as well as satiety signals including the duodenal peptide cholecystokinin- (CCK) (Woods & Strader, 2005), both of which have been

observed in human and animal studies (Woods & Langerhans, 2012). Satiety signals such as CCK are secreted from intestinal cells as food is digested and exits the stomach into the duodenum, with the signal aiding the digestive-absorptive process and has also been related to a reduction in meal size in rats (Gibbs, Young & Smith, 1973). These satiety signals, as well as directly influencing consumption also interact with adiposity signals, with the influence of these signals gradually increasing until the meal is terminated. In particular insulin and leptin alter the sensitivity to CCK increasing its action as a satiety signal to reduce consumption and meal size (Riedy, Chavez, Figlewicz & Woods, 1995). Insulin itself also appears to modulate food intake and weight maintenance, with administration in rats as well as mice reducing food intake (Brown, Clegg, Benoit & Woods, 2006; Woods, Chavez, Park, Riedy, Kaiyala et al, 1996) but also increasing food intake when substantial weight loss has already occurred (Chavez, Kaiyala, Madden, Schwartz & Woods, 1995).

To understand how such homeostatic mechanisms may influence short-term adaptation after consumption, the process of digestion in mice needs to be considered. In rodents the nucleus solitary tract (NTS) acts as the first central taste relay, receiving taste information via cranial nerves, and also projects to the rodent pontine taste area in the parabrachial nucleus (PBN) to alter and direct eating behaviour. Taste evoked activity in the NTS and the PBN has been found to be influenced by signals related to physiological state of the animal and satiety signals present. For example, Jacobs, Mark and Scott (1988) found that in sodium deficient rats which show an increased sodium preference, taste evoked reactivity alters in a manner consistent with an increase in the hedonic value of the sodium, which may aid

increased consumption and restoration of sodium levels. Taste evoked activity in the NTS has also been found to occur as a function of the concentration of a sucrose solution being consumed (Giza & Scott, 1987), suggesting that activity corresponds to relates to the perception of sweetness of the solution.

Furthermore, Giza and Scott (1983, 1987) found in rats that blood glucose concentration affected the taste evoked responding in the NTS, with glucose injections reducing responding by an average of 43% and consumption also decreasing as an inverse function of glucose concentration. Insulin also appears to modulate taste evoked responding in the NTS, with injections reducing taste evoked responding to glucose and fructose 7-22 minutes after receiving the insulin (Giza & Scott, 1987a, 1987b). Together these studies suggest that the presence of glucose and insulin, two satiety related signals, alter the taste evoked activity of the NTS in a way equivalent to reducing the concentration of the sucrose solution. This mechanism may therefore provide the means for the reduction in palatability and consumption during short-term adaptation, with the reduction in palatability seen in lick cluster size similar to that of an actual change in concentration (e.g. Dwyer, 2008). As this mechanism relies on absorptive effects, with glucose concentration rising 5-25 minutes after consumption with a subsequent slow rate of recovery, it may relate not to the immediate decline seen in short-term adaptation but rather a slightly longer adaptation effect such as that seen by Cabanac (1971). It therefore appears that although short-term memory may explain the adaptation seen shortly after consumption, homeostatic processes also play a part in regulating eating behaviour and should be considered when investigating such reduction in responding.

1.4 Overview

This thesis aims to investigate the short-term adaptation occurring after recent consumption of sucrose solution in mice. To investigate these changes in eating behaviour occurring after recent intake both the total number of licks and the mean lick cluster size, a measure of palatability, will be measured during each experiment. In particular the time course of the reduction in the total number of licks and mean lick cluster size (palatability) will be examined. This will be achieved by presenting short feeding opportunities in which the sipper tube containing 16% sucrose solution is available, with these opportunities separated by various intervals. These different intervals allow the duration and recovery period of the short-term adaptation occurring after consumption, both in total licks and mean lick cluster size, to be observed. During these experiments there was found to be an inverted U-shaped function of palatability (mean lick cluster size) with time since consumption.

This suggests that rather than being a single short-term adaptation effect that alters the palatability of a sucrose solution, there is also a secondary short-term process with a longer time course. The subsequent experiments therefore focused on investigating the mechanism behind this second decline in palatability, by ruling out two possible experimental confounds that may have previously caused the effect. Firstly the previous use of a within-subjects design and resulting conditioned frustration is tested, before the differing start times of the second feeding opportunity relative to the start of the session is also investigated.

Chapter Two

Time since consumption is an important factor regulating eating behaviour due to the influence of short-term adaptation, the change in responding seen after intake. This adaptation has been found to alter the hedonic response to a food as it is consumed, as well as in many instances being sensory-specific for foods recently experienced. The preference for a novel food over that recently consumed has been observed in a range of animal studies including rats (Rolls, Duijvenvoorde & Rowe, 1983; Young, 1940) and primates (Burton, Rolls & Mora, 1976; Rolls, 1981; Rolls, 1989). As well as this preference for other foods over that recently consumed, there is also some limited evidence for a reduction in palatability after consumption in rats (Berridge, 1991; Dwyer, 2012), supporting the idea that this may relate to the reduced motivation to consume seen during short-term satiety. Similarly, human studies have observed both a reduction in preference ratings and intake of foods post consumption (e.g. Havermans, Janssen, Giesen, Roefs & Jansen, 2009; Hetherington, Rolls & Burley, 1989) further supporting the reduced intake in short-term adaptation being related to altered palatability.

Although this short-term adaptation to recently consumed foods has been demonstrated in both human and animal studies, there are only very limited demonstrations of this short-term adaptation using lick cluster size as a measure of palatability. Dwyer (2012) found that in rats repeated presentations of a sweet solution resulted in a reducing mean lick cluster size using both high and low sucrose concentrations. Experiment one in this chapter will therefore demonstrate the

short-term adaptation in the form of reduced palatability (mean lick cluster size) and intake (total licks made) in mice after consumption of a sucrose solution.

Once it has been demonstrated that lick cluster size and total licks decline with recent consumption of sucrose, experiment two further investigated the influence of this effect by comparing relatively more massed or spaced schedules of access to sucrose, each presented over the same overall time period. If there is recovery of short-term adaptation to sucrose over brief intervals, then this should be seen in a weakened adaptation effect in the relatively spaced schedule in comparison to the more massed, in which the opportunity for recovery is reduced. These different levels of short-term adaptation may result in different levels of palatability and potentially a lower lick cluster size during the massed exposure when the recovery period is reduced.

A secondary aim of this experiment was to investigate if any changes in palatability of the sucrose solution occurring as result of the different schedules and levels of adaptation can be learned. If this is the case then the perceived sweetness of the solution and palatability of the solution presented, may alter depending of the schedule that is expected. One example of such a manipulation affecting consumption is the negative contrast effect, in which prior experience of a lower concentration sucrose solution subsequently reduces the palatability of a lower concentration solution than if there had been no such previous experience (e.g. Austen & Sanderson, 2016; Grigson, Spector & Norgren, 1993). To test if the mice learn about the different levels of palatability of the sucrose solution resulting from the differing schedules of access, these schedules were each presented in one of two distinct contexts. If any learning about the sucrose palatability occurs, then the association between the

context and the palatability may result in the context retrieving a representation of the perceived sweetness of the solution and subsequently altering consumption.

The short-term adaptation effect has been widely documented to be a sensory-specific process that occurs rapidly after consumption, demonstrated through food variety increasing intake within a meal. Furthermore, the duration of the effect also appears to support the argument that short-term adaptation after consumption is related to sensory processes (Berridge, 1991; Hetherington et al 1989). However there may also be a secondary influence of post-absorptive mechanisms that occur later after consumption, with maximal decline in pleasantness being reported up to an hour after consumption (Cabanac, 1971; Duclaux, Feisthaue & Cabanac, 1973). Experiment three therefore investigated the time course of the short-term adaptation to sucrose previously demonstrated in experiment one. This was achieved by presenting the mice with two separate short feeding opportunities, with three different intervals between these feeding opportunities. As these intervals increase in duration the effect of the short-term adaptation should weaken, resulting in recovery of palatability and mean lick cluster size during the second feeding opportunity.

To investigate a potential neural basis of the reduction in responding that occurs during the short-term adaptation effect, experiment three also tested GluA1 knockout mice. The GluA1 subunit of the AMPA receptor has been implicated in hippocampal synaptic plasticity (Erikson, Maramara & Lisman, 2010; Zamanillo, Sprengel, Hvalby, Jensen, Burnashev et al, 1999) and also short-term memory processes. Specifically, deletion of the GluA1 subunit has been found to result in impaired habituation, a decline in responding with exposure to recently experienced stimuli (e.g. Sanderson,

Hindley, Smeaton, Denny, Taylor et al, 2011; Sanderson, Sanderson & Bannerman, 2012). This finding of impaired short-term memory for recently experienced stimuli is in contrast to the spared associative memory also observed in the GluA1 knockout mice (Sanderson, Good, Skelton, Sprengel, Seeburg, Rawlins & Bannerman, 2009). Together these findings demonstrate that the GluA1 subunit is required for the expression of short-term memory for recently experienced stimuli, but not for associative memory mechanisms. Furthermore, short-term memory may be an important mechanism for the reduction in responding seen after consumption, with the stimulus representation resulting in habituation.

The effect GluA1 deletion on short-term habituation has been described in terms of Wagner's (1981) SOP model of memory (Sanderson, McHugh, Good, Sprengel, Seeburg, Rawlins & Bannerman, 2010). This model proposes that there are three states of memory in which a stimulus representation can be held, a primary active state (A1) a secondary active state (A2) and also an inactive state of memory. The level of behavioural responding to a stimulus is dependent on the state in which the representation is currently held. In particular responding is greatest when the stimulus representation is in the A1 state of memory, decreasing as it decays to the A2 state before decaying further to the inactive state, in which it no longer influences behaviour. Importantly once a representation has decayed to the A2 state in cannot return to the primary A1 state of memory until it has decayed back to the inactive state. This can explain the habituation of behavioural responding as being due to the stimulus representation having decayed to the A2 state, meaning that if the stimulus is presented again shortly after, responding will remain reduced. Therefore GluA1

deletion has been suggested to slow the decay rate of the stimulus representation between the primary (A1) and secondary (A2) active states of memory, resulting in the failure to habituate to recently experienced stimuli (Sanderson, McHugh, Good, Sprengel, Seeburg, Rawlins & Bannerman, 2010). The GluA1 subunit therefore provides a potential neural and psychological basis for the short-term adaptation that occurs with recent consumption. If the GluA1 knockout mice fail to show this reduction in intake and palatability, then this would demonstrate it to be dependent on the GluA1 subunit of the AMPA receptor.

This chapter will firstly demonstrate short-term adaptation to sucrose in mice before further investigating the influence of this on schedules of consumption in experiment two. Finally experiment three continued to investigate the time course of the adaptation over three different interval durations between sucrose solution feeding opportunities, with the prediction that increasing recovery will be seen as the interval between feeding also increases.

2.1: Experiment 1

This aimed to demonstrate short-term adaptation to a palatable sucrose solution in mice. Previous experiments in humans as well as animals have found that recent consumption reduces the hedonic response to the food, resulting in reduced consumption as well as palatability (Hetherington, Rolls and Burley 1989; Rolls, 1986). However the influence of such short-term adaptation in mice is unknown. To investigate the effect of recent consumption of a sucrose solution on palatability in mice, they were presented with a 16% sucrose solution for ten minutes and the lick cluster size as well as total number of licks measured. If palatability is affected by short-term adaptation that is seen with consumption, then this should be seen in the mean lick cluster size decreasing during the ten minutes of access to the sucrose solution and consumption.

Method

Subjects

Fifteen female C57BL/6J/Ola mice from Charles River UK were used. Mice were caged in a temperature-controlled housing room in groups of three to four, with a 12hr light-dark cycle (08:00-20:000). Mice were approximately four months old at the start of testing and weighed between 16.2 and 20.9g. They were maintained at 85% of their free feeding body weights with ad libitum access to water in their home cages for the duration of testing.

Apparatus

A set of eight identical operant chambers (interior dimensions: 21.6 x 17.8 x 12.7cm; ENV-307W, Med Associates) enclosed in sound-attenuating cubicles (ENV-022V, Med Associates) was used. The operant chambers were controlled by Med-PC IV software (Med Associates). The side walls were made from aluminium, and the front and back walls and the ceiling were made from clear Perspex. The chamber floors each comprised a grid of 24 stainless steel rods (0.32cm diameter), spaced 0.79cm apart and running perpendicular to the front of the chamber (ENV-307W-GFW, Med Associates). Retractable sippers (ENV-352AW, Med Associates) and a small hole in one wall of each chamber allowed graduated pipettes to be extended into, and retracted from, the chambers. The graduated pipette (0.1 ml) allowed measurement of consumption by comparing the volume before and after testing. Contact lickometer controllers (ENV-250, Med Associates) allowed contacts between the mice and the graduated pipettes to be recorded at a resolution of 0.01s. A fan (ENV-025F) was located within each of the sound-attenuating cubicles and was turned on during sessions. Sucrose solutions were made 16% weight/volume with commercially available sucrose in distilled water.

Procedure

Mice were given eight sessions in which the sipper tube containing 16% sucrose solution was inserted into the chamber for a duration of ten minutes. Each session lasted for a total of fifteen minutes with the sipper tube extended into the chamber after the first five minutes, with one such session a day.

Analysis

The criteria used to define a completed lick cluster were the same across experiments and similar to that previously used by Davis and Smith (1992), with licks separated by less than 500ms being defined as belonging to the same cluster of licks. This temporal interval is used due to the majority of licks that occur within clusters being separated by a duration equivalent to a single lick (approximately 150ms to 250ms), whereas the clusters themselves are separated by intervals from 500ms to many seconds in duration. (Davis, 1973; Davis & Smith, 1992) Therefore, a 500ms interval, timed from the end of one lick to the start of the next, should incorporate most of the completed clusters without increasing the chance of a second cluster being included within the previous.

For each experiment, three measures of licking behaviour were recorded: the total number of licks, the mean lick cluster size and the volume consumed. During each given time period the lick cluster size was calculated by dividing the total number of licks made within clusters within that time period by the number of completed lick clusters also made within that time period. In order to investigate the differences occurring within sessions, the values used to calculate the lick cluster size were averaged across sessions, resulting in an approximate mean lick cluster size for each animal during a given time period. During each session the sipper tube was inserted into the chamber only for the specified sipper duration, meaning that it may be retracted while the animal was making a cluster of licks. In this case the licks were still recorded and added to the total number of licks, but no completed cluster was recorded, therefore the approximate mean lick cluster size calculated here when only

completed clusters are included may differ than if the number of clusters started had been used.

The data in this and all subsequent experiments was analysed using either a one-way or a multifactorial ANOVA. Any interactions were analysed with simple main effects analysis using the pooled error term from the original ANOVA, or, for within-subject factors with more than two levels interactions, were analysed using separate repeated measures ANOVA. A Greenhouse-Geisser correction was applied when sphericity of within-subjects variables could not be assumed to produce more conservative p-values.

Results & Discussion

The data were split into ten 1-minute time bins corresponding to the ten minutes of access to the sucrose solution and averaged across the eight sessions, providing an average number of total licks and mean lick cluster size made during consumption. A repeated measures ANOVA of minute was carried out on the data.

Total Licks. The average number of total licks made during consumption of the sucrose solution decreased with minute during the 10-minute session as shown in the top panel of figure 1. The ANOVA showed a significant effect of minute F (9,126) = 131, $p < .001$ with a trend analysis showing a significant linear trend between minute and total number of licks, F (1,14) = 562, $p < .001$, as well as a significant quadratic trend, F (1,14) =16, p = .001).

Lick cluster size. The mean lick cluster size also showed a decrease with minute as shown in the lower panel of figure 1, with the greatest decline after 1-minute of access to the sucrose solution. The ANOVA showed a significant effect of minute F (9,126) =7.2, p <. 001 and a trend analysis showed a significant linear trend F (1,14) = 15, p =. 001 and no significant quadratic trend, F (1,14) = .032, p = .86.

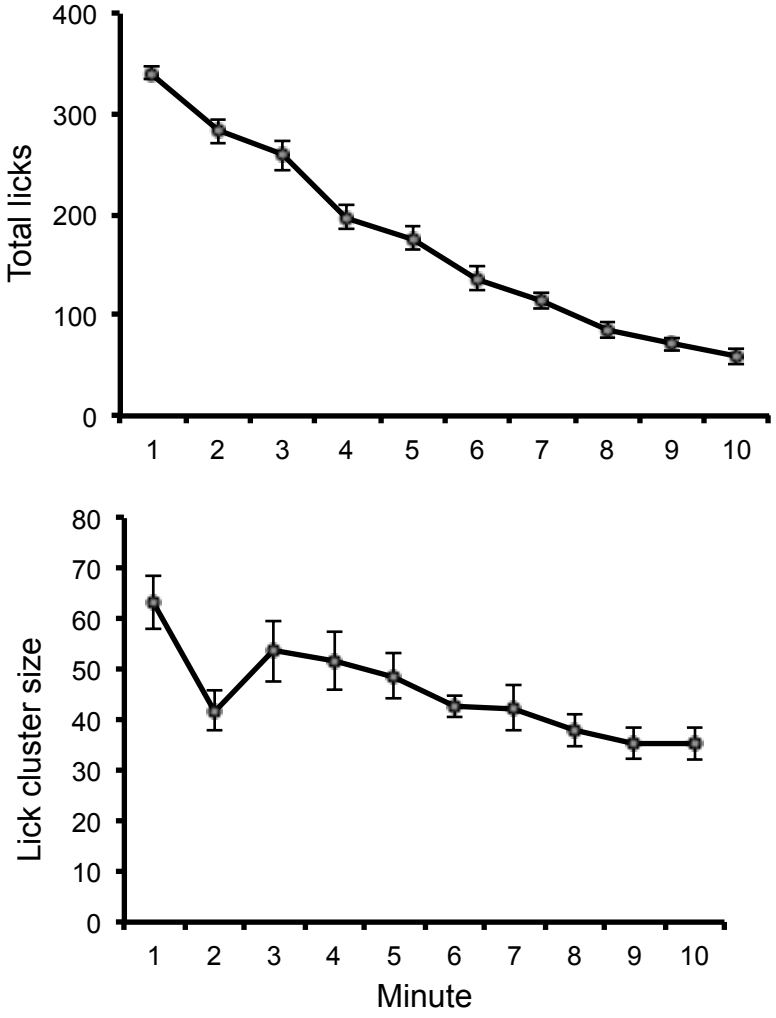

Figure 1 The average number of total licks (top) and mean lick cluster size (bottom) during consumption of a 16% sucrose solution, shown in one-minute time bins. Error bars show ± standard error of the mean.

Both the total number of licks and the mean lick cluster size made during consumption of the 16% sucrose solution declined during the ten minutes of access. This demonstrates the occurrence of short-term adaptation in the form of both reduced intake and a reduction in palatability with recent consumption of a sucrose solution in mice. This also supports previous experimental data in rats, in which short-term adaption occurs in the form of reduced consumption (Rolls, Duijvenvoorde & Rowe, 1983) and reduced palatability (Berridge, 1991; Dwyer, 2012). Furthermore, the greatest decrease in lick cluster size is seen after only 1-minute of previous consumption, suggesting that this adaptation occurs rapidly to reduce the palatability of the solution and supporting a sensory mechanism mediating the adaptation. As the linear reduction is the same as the function seen when the actual concentration of a sucrose solution is altered, the results also support the reduced palatability being related to a reduction in the perceived sweetness of the solution.

There are a variety of mechanisms, however, which may explain the short-term adaptation seen here with consumption of a sucrose solution. Fatigue, for example, would explain the reduction in both total licks and mean lick cluster size with continued consumption. Although the influence of this is hard to rule out, in this instance the mice were given access to a 16% sucrose solution and, as previously demonstrated, lick cluster size follows a linear function, with mean cluster size greater at higher concentrations (Davis & Smith, 1992; Dwyer, 2008). As the mean lick cluster size can be greater when consuming higher concentrations of sucrose, this questions the extent to which fatigue may have affected this particular measure in this current

experiment. The finding in rats however, that adaptation recovers with consumption of a new food (Berridge, 1991), provides some evidence that fatigue does not solely determine the adaptation effect seen.

Similarly, sensory adaptation could also explain the reduction in the total number of licks and reduced lick cluster size seen with consumption. Without demonstrating that intensity does not also correspondingly decrease, it cannot be ruled out as contributing to the effect. As well as fatigue and sensory adaptation, short-term memory processes may also be determining the decline in total licks and palatability, such as the decay of the stimulus representation in short-term memory (e.g. Wagner, 1981) resulting in a reduction in responding to the food being consumed.

2.2: Experiment 2

The first experiment demonstrated that both palatability (mean lick cluster size) as well as the total number of licks made during consumption of a 16% sucrose solution, decline with recent consumption, suggesting that the perceived sweetness of the solution is reduced with consumption. One potential effect of this is that the schedule of access to the sucrose solution may be an important factor in determining the palatability of a sucrose solution, due to it altering the strength of short-term adaptation effect. In particular, having fewer feeding opportunities of increased duration (massed schedule) should increase the influence of short-term adaptation compared to a higher number of shorter feeding opportunities (spaced schedule), in which there may be recovery of adaptation between feeding opportunities. If this is the case then palatability will be greater for the sucrose solution consumed during a spaced schedule than when the same solution is consumed in a more massed schedule, as any short-term adaption and corresponding decline in palatability resulting from previous consumption will have weakened.

Therefore, the first aim of this experiment was to investigate how different schedules of access to the same 16% sucrose solution used in the previous experiment, would alter the palatability during consumption in a 'massed' compared to a 'spaced' schedule of access. In the previous experiment 1-minute of access to the sucrose solution resulted in the greatest decline in palatability over the 10-minutes of access, demonstrating adaptation to occur rapidly with recent consumption. Therefore, to test the influence of this adaptation during different schedules of feeding, the spaced schedule consisted of ten 1-minute feeding opportunities each separated by a

recovery period of 4-minutes. The massed schedule consisted of two 5-minute feeding opportunities separated by a period of 36-minutes, equating the session lengths and time in the context for both schedules of feeding opportunity. If there is recovery of the short-term adaptation effect and decline in palatability with time since consumption, then the influence of this adaptation effect should be greatest in the more massed schedule, due to the 5-minute feeding opportunities allowing little time for recovery.

As well as short-term adaptation influencing palatability, there are also learning effects that can alter the palatability of the solution being consumed. One example of this is the negative contrast effect (e.g. Grigson, Spector & Norgren, 1993), in which previously receiving a higher concentration solution subsequently reduces the palatability of a lower concentration, than if only this lower concentration had been previously experienced. Furthermore this negative contrast effect has been found to be context dependent, for example Austen and Sanderson (2016) presented high and low sucrose solution concentrations in two different contexts, before presenting the low concentration in both contexts. It was found that when the high concentration had previously been experienced in the given context, the mean lick cluster size was reduced, an effect which was not seen when the low concentration had been previously experienced in the context.

Similarly, the different levels of palatability resulting from the schedules of access to the sucrose solution may also become associated with the context it occurs in. The second aim of this experiment was therefore to investigate if any differences in the perceived palatability of the sucrose solutions could be learned about. In order to test

this, each of the two schedules of access were presented in one of the two distinct contexts during training. This allowed for the different schedules and palatability of the sucrose solution to become associated with a distinct context. During the test sessions the mice were then presented with both of the massed and spaced schedules of access to the sucrose solution in both of the contexts. This will result in test sessions in which the schedule presented is the same as that previously received within that context during training, as well as sessions in which they are different. If there is an association between the context and palatability of the sucrose solution, then the context may retrieve a representation of this palatability, with this expectation potentially altering the mean lick cluster size during the incongruent test sessions.

Methods

Subjects & Apparatus

Eight female C57 mice (Charles River UK Ltd) were used and were approximately 11 weeks old at start of testing. Mice weighed between 14.0 and 16.9g and were caged in a temperature controlled housing room in groups of four, with a 12hr light-dark cycle (08:00-20:000). They were maintained at 85% of their free feeding body weights with ad libitum access to water in their home cages throughout testing. Further to the apparatus used in experiment 1, the requirement of two distinct contexts resulted in the addition of a house light (28V, 100mA; ENV-315M, Med Associates) and a clicker that clicked at a rate of twice a second (2Hz, 75dB; ENV-335M, Med Associates). The house light was located in the top centre of the wall directly opposite the retractable sipper tube and the clicker located left of the house light. Context A was created by having presentation of both the house light and clicker to create a bright and noisy

environment and context B was created through having neither of these cues presented resulting in a dark, quiet environment.

Procedure

Mice received ten sessions of training, one a day, in each of which they received both schedules of access in the paired contexts. For half of the mice context A (dark and quiet) was paired with the massed schedule and context B (bright and noisy) with the spaced schedule. For the other half of the mice this order was reversed. During each daily session the mice were placed in one context and the paired schedule presented, before being removed from the chambers and the volume consumed recorded. They were then placed back in the chamber and the second context and schedule of access given. The two contexts given in a daily session were presented in a repeating double alternating order across the ten training sessions, with context A then B given on session one before B then A given on session two (AB – BA – AB – BA). The massed schedule of exposure consisted of two 5-minute feeding opportunities to the 16% sucrose solution, separated by a 36-minute interval from the end of the first feeding opportunity to the start of the second. The spaced schedule consisted of ten 1-minute feeding opportunities to the sucrose solution, each separated by an interval of 4-minutes from the end of one to the start of the next. For both schedules of access mice had a 5-minute period in the chamber before the first feeding opportunity started by the sipper-tube being inserted into the chamber. In order to ensure that total time spent in each of the contexts were the same for each mouse, the two schedules were given over the same overall period of time.

Following these ten training sessions mice were given six days of test sessions, during which they received either congruent or incongruent pairings of context and schedule. In a congruent pairing the schedule was presented in the same context as during training, whereas for the incongruent the schedule is different to that given during training in that particular context. The two different contexts were presented in the same double alternating order as during training (AB on session one then BA on session two), resulting in half the mice receiving congruent before incongruent on test session one, then incongruent and congruent on test session two. For the other half of the animals this order was reversed. Across the six sessions each animal received three sessions of each context and schedule congruency pairing (congruent massed, congruent spaced, incongruent massed and incongruent spaced).

Results & Discussion

The training and test data for the massed and spaced schedules of feeding opportunities was split into two 5-minute time bins and averaged over sessions, with this being the duration of the feeding opportunities in the massed schedule of feeding to compare to the spaced. The training data was analysed using a schedule x bin ANOVA and for the test data a training context x test schedule x time bin ANOVA was carried out. A main effect of training context would show that previous experience of the context with either a massed or spaced schedule influences eating behaviour. A main effect of test schedule would demonstrate that the schedule presented influences eating behaviour.

Training

Total licks. The total number of licks made during the massed and spaced schedules, averaged across the ten training sessions, for the first and second 5-minute time bins are shown in the top panel of figure 2. For the massed schedule of feeding the total number of licks remained stable across the two 5-minute time bins. The spaced schedule showed a slightly greater number of licks than the massed schedule in the first 5-minutes which subsequently decreases below the massed schedule in the second five minute time bin. The ANOVA showed there was a significant main effect of 5-minute time bin, F (1,7) = 18, p = .003, but no significant main effect of feeding schedule, F (1,7) = .20, p = .66. There was also a significant interaction between feeding schedule and bin, F (1,7) = 14, p = .006. Simple main effects analysis of the interaction showed that for the first 5-minute time bin the spaced schedule had a greater total number of licks that neared significance, F (1,7) = 5.1, p = .057, whereas in the second 5-minute time bin the total number of licks was greater for the massed schedule F (1,7) = 8.5, p = .02. For the spaced feeding schedule the total number of licks were greater in the first 5-minute time bin compared to the second, F (1,7) = 19, p = .003, but not for the massed schedule, F (1,7) = .38, p = .55.

Lick cluster size. The mean lick cluster size made during consumption in the massed and spaced schedules, averaged across the ten training sessions, for the first and second 5-minute time bins are shown in the middle panel of figure 2. The spaced feeding schedule had a greater lick cluster size for the first 5-minute time bin compared to the massed schedule, with both schedules decreasing between the first and second 5-minutes to a similar mean lick cluster size. The ANOVA showed that

there was no significant main effect of 5-minute time bin on lick cluster size F (1,7) = 1.6, p = .23 and a main effect of feeding schedule that approached significance F (1,7) = 4.5, p = .070 and no interaction between these factors F (1,7) = .27, p = .61.

Consumption. The total volumes consumed, averaged over the training sessions, is shown in the bottom panel of figure 2, with no difference in the amount consumed for the massed and spaced schedules. The ANOVA showed there was no significant main effect of feeding schedule on the volume consumed during training, F (1,7) = .34, p = .57.

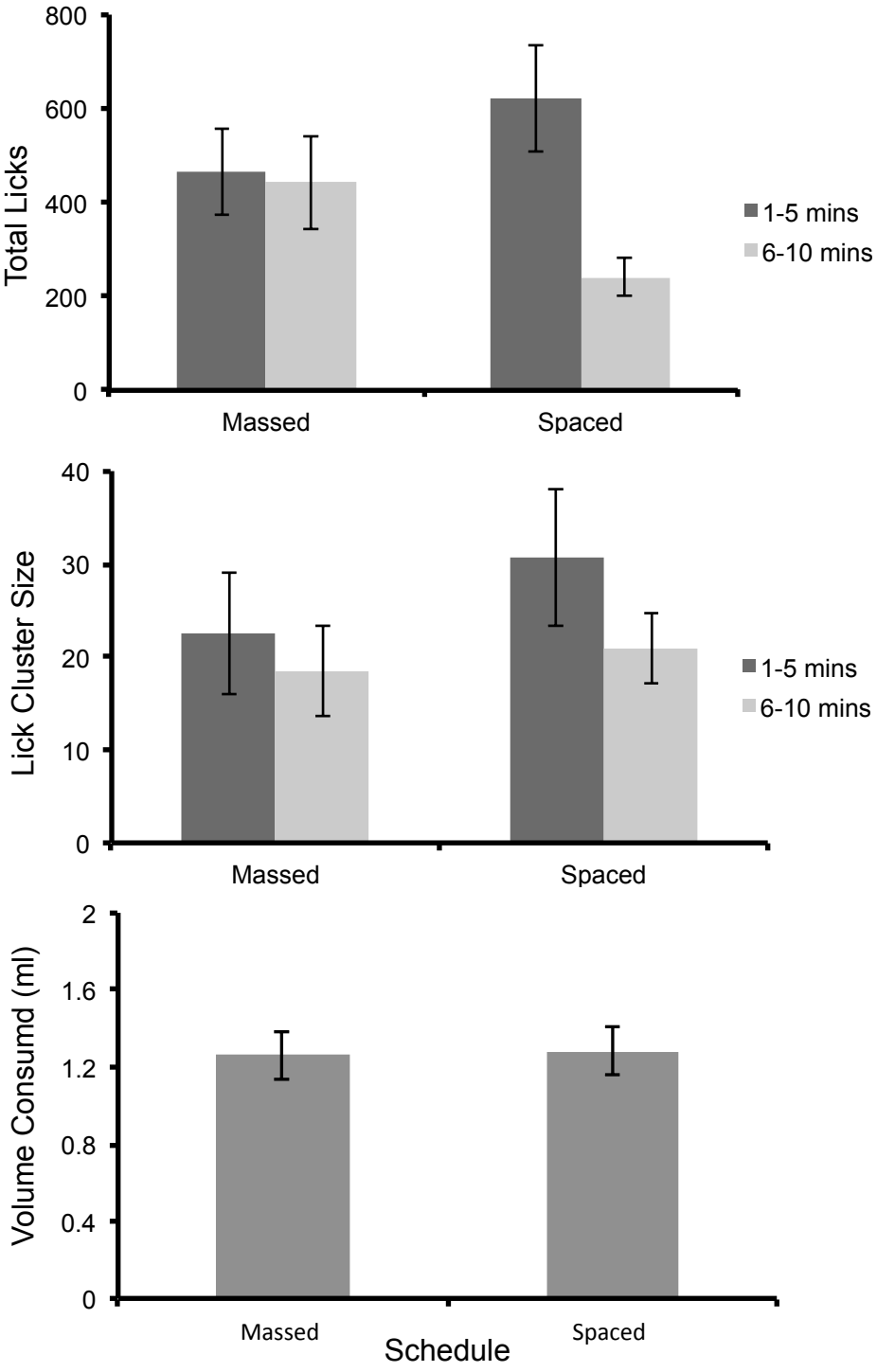

Figure 2. The average number of total licks (top) and mean lick cluster size (middle) made during consumption of a 16% sucrose solution, in the first and second 5-minute time bins during training. Lower panel shows volume consumed during training. Error bars show ± standard error of the mean.

Test

Total licks. The total number of licks made during consumption in the first and second 5-minutes, averaged across the three test sessions for each congruency condition, is shown in the top panel of figure 3. All congruency conditions showed a higher number of total licks during the first 5-minute time bin compared to the second, with a slightly larger number of licks made during the spaced schedule than the massed schedule sessions. The ANOVA showed a significant main effect of 5-minute time bin F (1,7) = 98, $p < .001$, showing that the total number of licks was greater during the first 5-minute time bin compared to the second 5-minutes for each feeding schedule. There was no significant main effect of schedule previously received in the context during training F (1,7) = .034, p = .85, showing that previous training within the context did not affect the total number of licks during consumption. There was also no significant main effect of feeding schedule presented during test, F (1,7) = .503, p = .501, showing that the total number of licks was not altered by the schedule presented during the test sessions. There were also no significant interactions, $F < 3.6$, $p > .098$.

Lick cluster size. The mean lick cluster size made during consumption, averaged across the three test sessions for each condition are shown in the middle panel of figure 3. Lick cluster sizes were similar across conditions for the second 5-minute time bin which were lower compared to the first 5-minute time bin. The spaced test schedule showed a greater lick cluster size than the massed, for both the congruent and incongruent pairings of training and test feeding schedule presented in the context, particularly in the first 5-minute time bin. The ANOVA showed a significant main effect of feeding

schedule presented during test *F* (1,7) = 7.8, p = .027, meaning that the actual schedule presented does alter palatability, in this case the spaced feeding schedule has a higher mean lick cluster size. The main effect of 5-minute time bin approached significance *F* (1,7) = 4.8, p = .063 and there was no significant main effect of training feeding schedule previously presented in the context *F* (1,7) = 1.04, p = .34, showing previous training did not influence mean lick cluster size. There was a significant test schedule x 5-minute time bin interaction *F* (1,7) = 5.7, p = .048, all other interactions were non-significant, F values < 2.08, p values > .19. Simple main effects analysis of the interaction showed that for the first 5-minute time bin the mean lick cluster size was greater during the spaced schedule of access than the massed schedule, *F* (1,7) = 7.4, p = .029. This was not seen in the second 5-minute time bin *F* (1,7) = .21, p = .21. Lick cluster sizes were also slightly higher during the spaced schedule for the first 5-minute time bin compared to the second 5-minute time bin which approached significance, *F* (1,7) = 5.3, p = .053, whereas there was no difference between the two time bins for the massed schedule of feeding opportunity *F* (1,7) = .60, p = .46.

Consumption. The average volume consumed is shown in the lower panel of figure 3, with all volumes similar across test conditions. The ANOVA showed that there were no significant main effects of feeding schedule presented during training in the context *F* (1,7) = .04, p = .84 or test feeding schedule *F* (1,7) = .30, p = .59, and no interaction between these factors, *F* (1,7) = .39, p = .55.

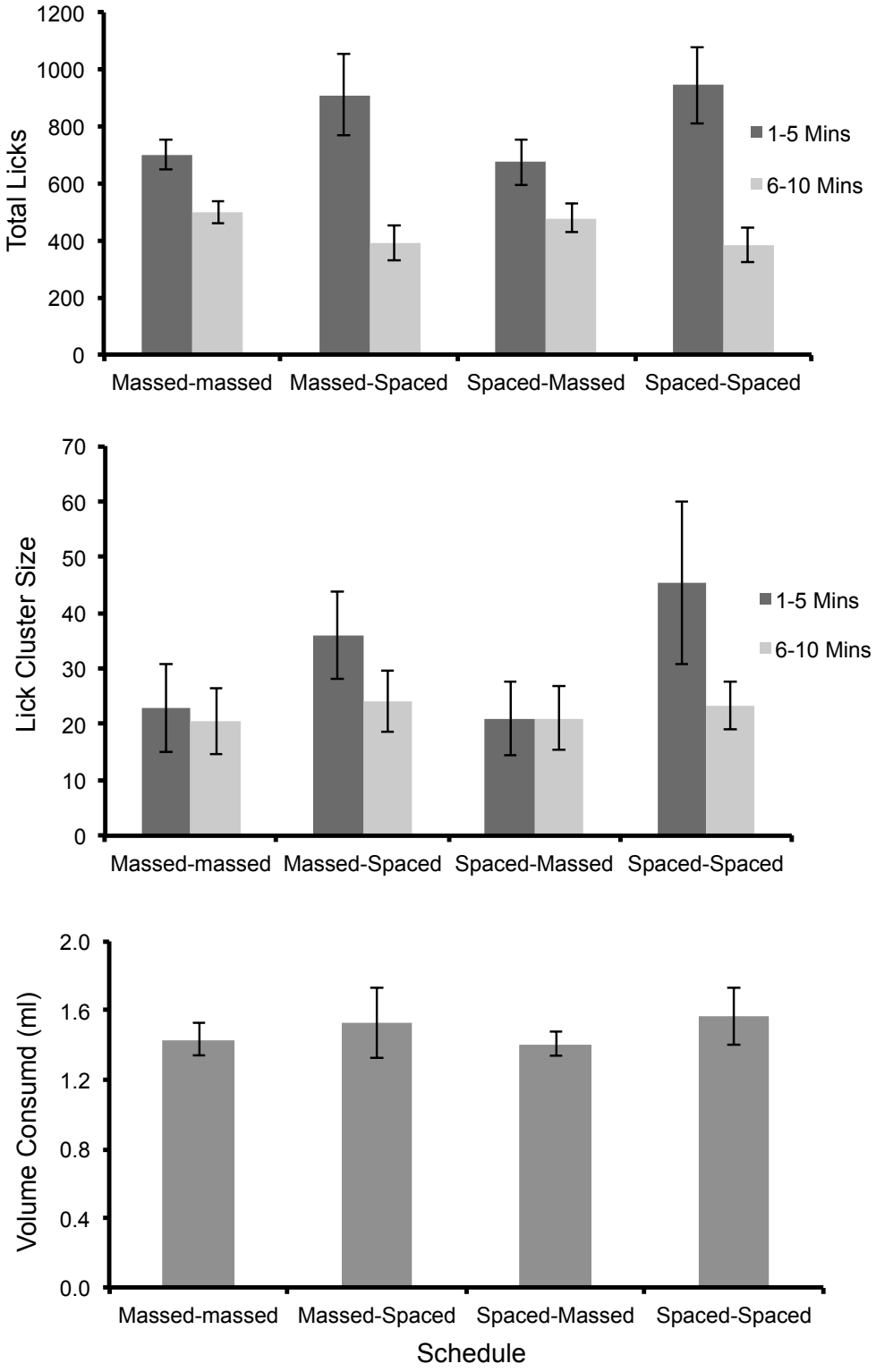

Figure 3. Average number of total licks (top) and mean lick cluster size made (middle) during consumption of the sucrose solution in the first and second 5-minute time bins during congruent and incongruent test sessions. Lower panel shows the average volume consumed during congruent and incongruent test sessions. Error bars show ± standard error of the mean.

Palatability (the mean lick cluster size) is affected by the different schedules of feeding opportunities with lick cluster sizes being larger for the spaced schedule, specifically in the first 5-minute time bin during both training and test sessions. This demonstrates that receiving a relatively more massed 5-minute long feeding opportunity reduces the palatability of the sucrose solution, compared to when the same total duration of feeding is spread over separate 1-minute feeding opportunities. Total licks however are not significantly affected by the schedule presented, although the first five minutes do have a beneficial effect on the total number of licks compared to the second, for both massed and spaced schedules of exposure during training and test. This finding that the average number of total licks does decrease with recent consumption, is in line with the results from the previous experiment and the occurrence of short-term adaptation resulting in a reduction in the total number of licks and the mean lick cluster size.

Although the test schedule did affect the palatability of the sucrose solution, there was no effect of the training schedule previously received in the same context. This suggests that palatability is more determined by the actual schedule received, either massed or spaced, rather than previous experience determining how palatable the solution is. It could also be the case that the animals failed to associate the distinct contexts with the schedules of feeding opportunities, meaning the context was unable to retrieve a representation of the perceived sweetness of the solution and alter palatability during consumption. However it is worth noting that the two distinct contexts used here were the same as during the negative contrast experiment by Austen and Sanderson (2016), in which the effect was found to be context dependent.

Experiment one demonstrated that short-term adaptation occurs quickly after consumption, with the greatest decline in palatability occurring after 1-minute of access and a general decline over the 10-minutes of access to a 16% sucrose solution. In this second experiment giving a spaced schedule of 10-minutes of feeding opportunity resulted in a greater palatability of the sucrose solution compared to a more massed schedule, in which there is the same overall feeding opportunity time but spaced only over two 5-minute periods of access. This difference in palatability can be explained through short-term adaptation to the sucrose having a lesser influence on the spaced than the massed schedule, due to recovery occurring in the 4-minute intervals between feeding opportunities and increasing palatability as a result. Therefore this experiment suggests that as well as the onset of the short-term adaption to sucrose after consumption being rapid, recovery can also occur within a short time frame, in this case within a period of 4-minutes. In order to understand the time-course of this adaptation effect further, experiment three investigated the reduction in palatability seen during short-term adaptation to sucrose over different feeding opportunity intervals.

2.3: Experiment 3

Experiment two previously showed that the short-term adaptation that occurs with recent consumption of sucrose reduces over a short 4-minute period between feeding opportunities. The aim of this experiment was to further investigate the time course of the short-term adaptation effect to a 16% sucrose solution in mice, specifically examining how long it takes for palatability to recover after one minute of access to the solution, a duration which previously showed the greatest subsequent decline in palatability. In order to investigate this the mice were twice presented with 1-minute long feeding opportunities in each session, for which the 16% sucrose solution could be consumed by the sipper-tube being inserted into the chamber. These two feeding opportunities were separated by one of three interval durations, 5 seconds, 10 minutes or 60 minutes.

As the short-term adaptation effect on palatability occurs rapidly after only a 1-minute period of access and also appears to recover quickly, then the influence of this adaption during the second feeding opportunity should decrease with increasing interval duration. It could therefore be expected that palatability will be lowest after the shortest 5-second interval with recovery after a 10-minute interval. The final 60-minute interval was used to ensure that the full time course of the adaption effect was seen, with this long interval allowing time for full recovery of the short-term adaptation and corresponding reduction in palatability and intake.

As well as the time course of the short-term adaptation effect and its recovery, this experiment also aimed to investigate the role of the GluA1 subunit of the AMPA receptor, a potential neural substrate for the reduction in responding seen during

short-term adaptation. The GluA1 subunit is important for hippocampal synaptic plasticity (Erikson, Maramara & Lisman, 2010; Zamanillo, Sprengel, Hvalby, Jensen, Burnashev et al, 1999) and has also been implicated in short-term memory. In particular, GluA1 knockout mice fail to show the reduction in responding normally seen with recent exposure to a stimulus (e.g. Sanderson, Hindely, Smeaton, Denny, Taylor et al 2011; Sanderson & Bannerman, 2012) therefore providing a potential neural and psychological basis for the reduction in both mean lick cluster size and the total number of licks that is seen after recent consumption.

Methods

Subjects & Apparatus

Twelve GluA1 knockout mice (six males, six females) and twelve wild-type (six males, six females) bred in the life sciences support unit at Durham University were used. The mice were between five and seven months old at the start of testing and weighed between 15.8g and 26.4g. They were caged in groups of one to five in a temperature controlled housing room with a 12hr light-dark cycle. During testing they were maintained at 85% of their free feeding body weights with ad libitum access to water in their home cages. The apparatus was the same as that used in experiment one.

Procedure

Mice were presented with two 1-minute long feeding opportunities per session in which the sipper tube was inserted into the chamber giving them access to the 16% sucrose solution, with one such session per day for fifteen sessions in total. The first feeding opportunity occurred after a 5-minute period in the chamber and the duration

of the interval between the two feeding opportunities was one of three durations from the end of one feeding opportunity to the start of the next. These durations were a short, 5-second interval, a medium 10-minute interval or a long 60-minute interval between feeding opportunities. The session order was randomised with the constant that on any given session one third of the mice each received one of the three different feeding opportunity intervals. Over each block of three days each animal received a session of each interval, with five such blocks given over the fifteen days.

Results & Discussion

The data was split into two 1-minute time bins corresponding to the two feeding opportunities. A mixed model ANOVA of feeding opportunity interval x feeding opportunity x genotype was carried out on the data, which was averaged over the five sessions of each interval duration for each animal. If differing amounts of time since consumption affect palatability then this will be seen in a significant main effect of opportunity interval, with any reduction in responding during the second feeding opportunity seen in a main effect of feeding opportunity.

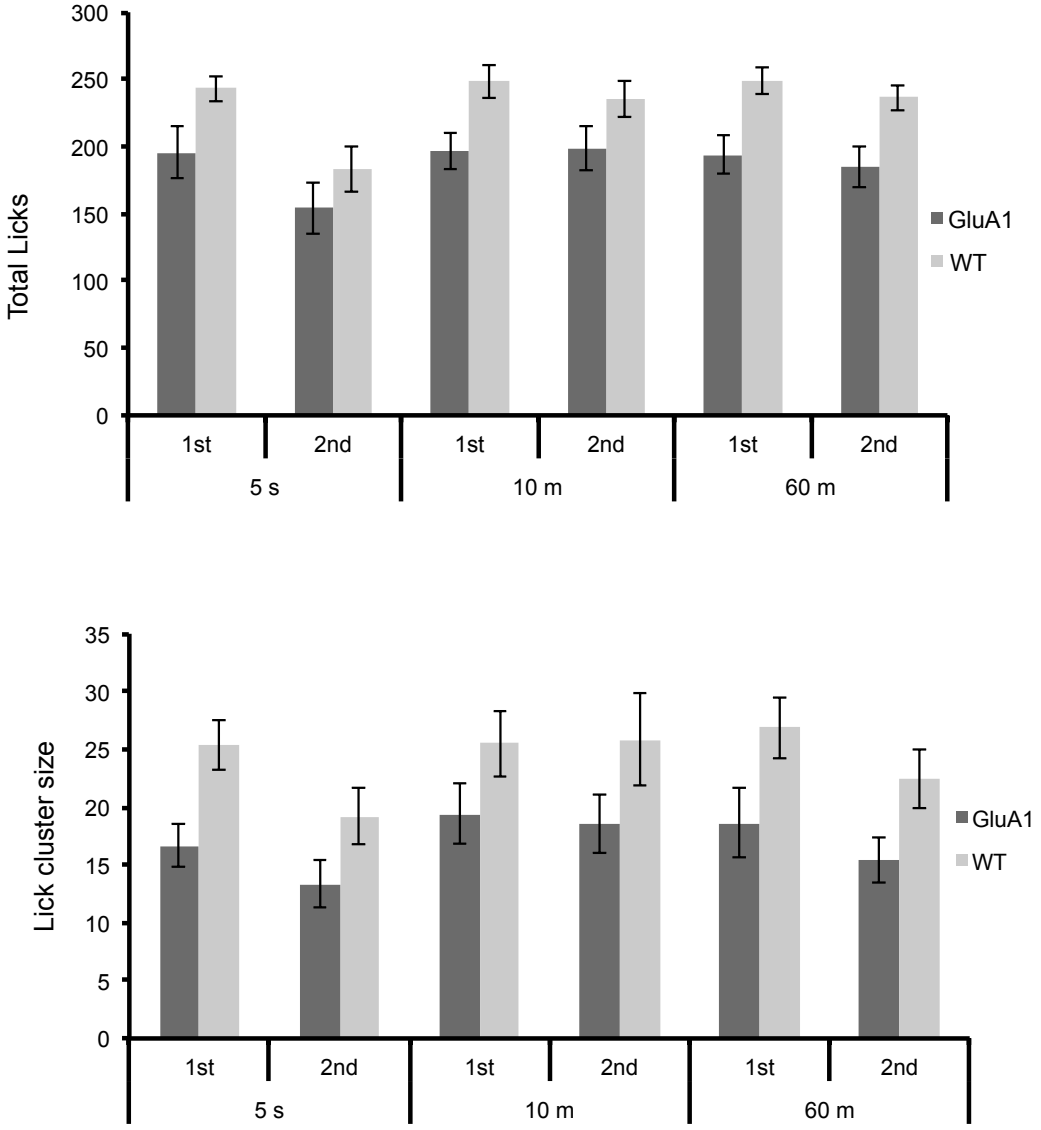

Figure 4. The total number of licks (top) and mean lick cluster size (bottom) made during consumption in the first and second feeding opportunities, for the wild type and GluA1 knockout mice for the 5-second, 10-minute and 60-minute intervals. Error bars show ± standard error of the mean

Total Licks*:* The average number of total licks made during consumption by the wild type and GluA1 knockout mice in the first and second 1-minute feeding opportunities before and after the three feeding intervals, are shown in the top panel of figure 4. The wild type mice show a greater number of total licks than the knockouts across all three intervals, but both show the same reduction after a 5-second interval that is not seen after a 10-minute or 60-minute interval. The ANOVA showed that there was a significant main effect of feeding opportunity interval, F (2, 44) = 9.1, p = .002, meaning the total number of licks is significantly affected by the time since previous consumption of sucrose. There was also a significant main effect of feeding opportunity, F (1,22) = 57.86, p <. 001 showing that the first and second 1-minute long feeding opportunities differ in the total number of licks made during consumption. There was also a significant main effect of genotype F (1,22) = 6.1, p = .021, showing the GluA1 knockouts make a significantly lower number of licks during consumption.

There was also a significant interaction between 1-minute feeding opportunity and genotype, F (1,22) = 4.8, p =. 039 as well as between feeding opportunity interval and feeding opportunity F (2,44) = 20, p <. 001. There was no interaction between feeding opportunity interval, feeding opportunity and genotype F (2,44) = .42, p = .64. Simple main effects analysis of the 1-miniute feeding opportunity x genotype interaction showed that the GluA1 knockout mice had a significantly lower number of total licks than the wild types in the first feeding opportunity F (1,22) = 8.8, p = .007 and near significantly lower in the second opportunity, F (1,22) = 3.9, p = .060. The GluA1 knockout mice also had a significantly lower number of total licks in the second feeding opportunity compared to the first F (1,22) = 14, p = .001, an effect which the wild types

also showed F (1,22) = 48, p <. 001. To further investigate the feeding opportunity interval x 1-minute feeding opportunity interaction, a repeated measures ANOVA of interval was carried out. This showed that for the first feeding opportunity there was no significant main effect of interval between feeding opportunities F (2,46) = .098, p =. 86. A repeated measures ANOVA of interval for the second feeding opportunity, showed that there was a significant main effect of interval F (2,46) = 20, p <. 001. Post hoc analysis using the bonferroni correction for multiple comparisons showed that the total number of licks were significantly lower after a 5-second than a 10-minute feeding opportunity interval (p <. 001) and a 60-minute interval (p =. 001) with no significant difference between the 10 and 60 minute intervals (p =. 83). Simple main effects analysis of the feeding opportunity interval x 1-minute feeding opportunity interaction, showed that the total number of licks was significantly greater in the first feeding opportunity compared to the second for the 5-second interval between feeding opportunities, F (1, 22) = 58, p <. 001 as well as for the 60-minute interval, F (1,22) = 5.1, p =. 033, but there was no difference for the 10-minute interval F (1,22) = 1.3, p =. 25.

Lick cluster size: The mean lick cluster size made during consumption for the wild type and the GluA1 knockout mice across the three feeding opportunity intervals and for the first and second feeding opportunities, is shown in the lower panel of figure 4. The wild type mice show a larger mean lick cluster size than the knockouts after all three feeding opportunity intervals, however both demonstrate a similar pattern of reduction after both the 5-second and 60-minute intervals that is not seen after a 10-minute interval. The ANOVA showed that there was a significant main effect of

feeding opportunity interval, *F* (2,44) = 7.3, p = .002, meaning that mean lick cluster size is significantly affected by time since previous consumption. There was also a significant main effect of 1-minute feeding opportunity *F* (1,22) = 23, p <. 001, meaning that the first and second feeding opportunities significantly differ, as well as a significant effect of genotype *F* (1,22) = 4.5, p = .045. There was also a significant interaction between feeding opportunity interval and 1-minute feeding opportunity *F* (2,44) = 7.29, p = .002, all other interactions were non-significant, *F* values < 1.2, p values > .28.

To investigate the feeding opportunity interval x 1-minute feeding opportunity interaction, a repeated measures ANOVA of interval on the first feeding opportunity showed that there was no significant main effect of feeding opportunity interval, *F* (2,46) = 1.7, p = .19. A repeated measures ANOVA on the second feeding opportunity showed that there is a significant main effect of interval *F* (2,46) = 11, p <. 001. Post hoc analysis of the effect of feeding opportunity interval using the bonferroni correction for multiple comparisons, revealed the 5-second interval duration had a significantly lower lick cluster size than 10 minutes (p = .002) and approached significance for the 60-minute interval p = .059). The 60-minute interval also had a significantly lower lick cluster size than 10 minutes (p = .025). Simple main effects of the feeding opportunity interval x feeding opportunity interaction further showed that the mean lick cluster size was significantly larger in the first feeding opportunity than the second for the 5-second interval, *F* (1,22) = 21, P <. 001 as well as for the 60-minute interval F (1,22) = 19, p < .001, but there was no significant difference between feeding opportunities for the 10-minute interval, *F* (1,22) = .067, P = .79.

There is a decline in palatability (mean lick cluster size) as well as total licks, after a very short 5-second interval between feeding opportunities. This is in line with the findings from the previous two experiments, in which short-term adaptation occurs rapidly with consumption and after only a brief 1-minute period of access to the sucrose solution. After a 10-minute interval between feeding opportunities the influence of any short-term adaption has weakened so that there is no change in palatability between feeding opportunities. Again this finding supports the results from experiment two, in which there is some recovery of the reduction in palatability after a period of 4-minutes between feeding opportunities. This suggests that an interval of only 10-mintues between two relatively short feeding opportunities, is sufficient for complete recovery of the short-term adaptation effect that occurs after recent consumption of sucrose.

However not only is there evidence for this expected adaptation process after consumption, there is also a secondary decline in palatability after a 60-minute interval between feeding opportunities. This is surprising, as it was expected that the recovery of the adaptation effect would continue with increasing time since consumption of the sucrose solution. Therefore rather than there being a single linear short-term adaptation effect, there appears to be an inverted U-shaped function of time since consumption on palatability, with reductions after both 5-second and 60-minute intervals. Furthermore, there was recovery of the first adaptation effect after a 10-minute interval for both the total number of licks and the mean lick cluster size. This suggests that process underlying this secondary decline is not due to the same

adaptation process resulting in the decline after only 5-seconds, but due to a secondary mechanism that also influences palatability.

This inverted U-shaped function of time since consumption on palatability was seen in both the wild type and the GluA1 knockout mice, meaning that neither the first short-term adaptation effect or the later secondary decline in palatability are GluA1 dependent. This finding is in contrast to previous research in which deletion of the GluA1 subunit results in a failure to habituate to recently experienced stimuli (e.g. Sanderson & Bannerman, 2012). However GluA1 deletion does seem to alter eating behaviour in the form a general reduction in responding, in this case in both the total number of licks and the mean lick cluster size. As a reduction in mean lick cluster size indicates a reduction in the perceived sweetness and palatability of the sucrose solution (e.g. Dwyer, 2012; Harris & Thein, 2005), it appears that the GluA1 knockout mice consume the sucrose in a way suggesting it to be less palatable than for the wild type mice. This suggests that the GluA1 deletion results in a general reduction in the hedonic value of sucrose solution, a finding in line with previous research suggesting GluA1 deletion may provide a model of anhedonia (e.g. Barkus, Feyder, Graybeal, Wright, Wiedholz et al, 2012; Fitzgerald, Barkus, Feyder, Wiedholz, Chen, 2010). Such a reduction in the hedonic value may explain the reduced palatability and intake seen in this experiment, with the lower total number of licks related to the reduced palatability of the sucrose solution.

2.4: Discussion

The first experiment demonstrated a decline in the mean lick cluster size and the total number of licks with consumption of a palatable 16% sucrose solution, showing that short-term adaptation occurs in mice in the form of reduced palatability and intake. This result supports previous findings in rats, that recent consumption reduces intake of the particular food consumed and may also reduce the palatability of the food consumed (Berridge, 1991; Dwyer, 2012). The second experiment further investigated the influence of this short-term adaption effect, finding a more spaced schedule of access to sucrose solution increases the palatability of the sucrose. This finding of recovery over short durations was further found in the third experiment, in which a 10-minute interval between feeding opportunities was sufficient for palatability and intake to recover.

Together these results support there being a short-term adaptation effect that occurs rapidly after recent consumption of sucrose solution. Furthermore, as well as this adaptation process having a rapid onset, it can also recover quickly, meaning it may act to control eating behaviour over relatively short time periods. Experiment three further found that this adaptation effect does not depend on the GluA1 subunit of the AMPA receptor, which has been implicated in short-term memory for recently experienced stimuli (e.g. Sanderson, Hindely, Smeaton, Denny, Taylor et al 2011). This suggests that the reduction in responding seen to sucrose may be due to a different process than the GluA1 dependent habituation process that occurs after recent exposure to other spatial and non-spatial stimuli.

However, as well as this quick-acting short-term adaptation process, the third experiment found a surprising second reduction in the mean lick cluster size after a 60-minute interval, that was not seen in the total number of licks. Importantly this suggests that there is not just a single process of short-term adaptation affecting eating behaviour after recent consumption. Rather, there may also be a second process altering palatability later after consumption. The recovery of the short-term adaptation after a 10-minute interval also indicates that the two reductions in palatability after 5-second and 60-minute intervals, each relate to different mechanisms. This finding of an inverted U-shape function of palatability with time since consumption was unexpected and if exists would have important implications for understanding short-term adaptation after consumption. The subsequent experiments therefore continue to investigate this second decline in palatability, to further test if there are two adaptation processes occurring after recent consumption of sucrose solution.

Chapter Three

The previous three experiments demonstrated that there is a short-term adaptation process that occurs with recent consumption, acting to reduce the palatability of the sucrose solution being consumed as well as the total number of licks made during consumption. This initial short-term adaptation occurs rapidly after consumption, seen after only 1-minute of access to the sipper-tube containing the 16% sucrose solution. However this adaptation effect can also recover quickly, with no such decline in palatability or the total number of licks after a 10-minute interval between the 1-minute feeding opportunities. As well as this initial decline in palatability, experiment three also found that the mean lick cluster size shows an inverted U-shape function with time, declining again after a 60-minute interval between feeding opportunities. This second decline after previous recovery of the initial short-term adaptation effect suggests that there is a second process affecting palatability after recent consumption.

The subsequent experiments aimed to investigate the cause of the secondary decline in palatability that occurs 60-minutes after consumption, by examining two potential experimental confounds which may have resulted in the effect. One potential cause of this second decline in palatability relates to the previous use of a within-subjects design during experiment three, resulting in each animal receiving all three of the different intervals between feeding opportunities, 5-seconds, 10-minutes and 60-minutes. As a result of this the mice may have come to expect a second feeding opportunity after the first, with the 60-minute interval resulting in a greater waiting

period than either the 5-second or 10-minute intervals. This increased time in the context, when the sucrose solution is expected but not presented, could cause negative prediction error and the occurrence of frustrative non-reward, resulting in an associated negative emotional state. If the animal is in such an emotional state, then this may reduce the palatability of the sucrose solution when it is presented after the full 60-minute interval.

As well as frustrative non-reward, another potential factor resulting in the secondary decline in palatability after 60-minutes is the differing start times of the second feeding opportunity relative to the start of the session. In the previous experiments the time spent in the chamber before presentation of the sucrose solution after an interval was not equated, meaning that longer durations between feeding opportunities were confounded by increased time in the context. It could therefore be the case that the reduced palatability seen during consumption of the sucrose solution after 60 minutes, may be due not to a process directly relating to previous consumption, but rather due to the additional time spent in the chamber. The final experiment aimed to investigate this by equating the time in the chamber before the second feeding opportunity was presented. If the second decline in palatability after a 60-minute interval remains despite controlling for conditioned frustration and time in the context, then this would provide further evidence for two short-term adaptation processes occurring after recent consumption of a sucrose solution.

3.1: Experiment 4

One potential explanation for the second decline in palatability seen after a 60-minute interval between feeding opportunities, is that it may have been caused by the within-subjects design used in experiment three. In this previous experiment each animal received all three feeding opportunity intervals, 5-seconds, 10-minutes and 60-minutes, in an intermixed order. It is well documented that animals can learn about schedules of food reinforcement, with responding occurring as a function of time and reaching its maximum around the time that reinforcement is expected to occur (e.g. Church & Broadbent, 1990; Gibbon & Church, 1982). One result of this is that the animals may have come to expect a second feeding opportunity to occur in each session, which for the majority of the time occurs after either a 5-second or a 10-minute interval. On one third of occasions however, this waiting period is extended to 60 minutes in duration. During this period any expectation of a second feeding opportunity being due to occur could result in negative prediction error, due to the animal expecting but not receiving the sucrose solution in the time period.

Frustration theory (Amsel, 1962; 1992) proposes that discrepancy between reward expectation and the reward received results in a primary frustration reaction, or frustrative non-reward, with the size of the discrepancy determining the strength of the frustration response. This frustration response acts to energise behaviour. For example Amsel and Roussel (1952) in a runway experiment found that the latency to reach a second goal box was decreased when the first goal box was non-rewarded. Furthermore, frustrative non-reward has also been associated with an aversive state, as rodents learn to avoid cues that elicit it (Daly, 1969) and can also result in learned

conditioned frustration (Wagner, 1963). Such frustration has also been used to explain the negative contrast effect (e.g. Bower, 1961), in which the downshift in reward may result in a frustration response acting to reduce responding to the smaller reward as a result. Further evidence for a role of frustration in the negative contrast effect comes from corresponding increases in plasma corticosterone, a hormone related to stress responses (Goldman, Coover & Levine, 1973).

In the case of a reduction in sucrose concentration (Austen & Sanderson, 2016; Grigson, Spector & Norgren, 1993) the failure of the more palatable higher concentration sucrose solution to be presented may result in a frustration response and reducing the palatability during subsequent consumption. Similarly, the inverted U-shape function of palatability that occurs with time since recent consumption may also be due to frustrative non-reward. In particular the expectation of a second feeding opportunity shortly after the first, may result in a frustration response during the 60-minute interval in which the sucrose solution fails to be presented. In turn this may result in a negative emotional state and reduction in the palatability of the sucrose solution when it is subsequently consumed. This should only occur however, when a within-subjects design is used, as this allows each animal to experience the different intervals between feeding opportunities and generate an expectation that the second feeding opportunity can occur before 60 minutes. Without such an expectation, there should be no discrepancy between expected and received reward.

The use of a between-subjects design would ensure that each animal, although may still come to expect two feeding opportunities in each session, would only ever expect the second to occur after a fixed interval of time. Therefore those in the 60-minute

interval condition should not expect the second opportunity to occur much before the 60-minute interval, meaning there should also be no corresponding frustrative non-reward and decline in palatability during consumption. This experiment will therefore test if the previous decline in palatability seen after a 60-minute interval is due to the previous use of a within-subjects design, which may have resulted in conditioned frustration reducing the palatability. Both the 10-minute and 60-minute intervals between feeding opportunities will again be given, however half of the mice will each receive only one of these different intervals over sessions.

Methods

Subjects & Apparatus

Twenty-four female C57BL/6J/Ola mice from Charles River UK were used. They were approximately ten weeks old at the start of testing, weighing between 17.1g- 20.8g. Mice were caged in groups of four in a temperature controlled housing room with a 12hr light-dark cycle. During testing they were maintained at 85% of their free feeding body weights with ad libitum access to water in their home cages. Apparatus was the same as used in the previous experiment.

Procedure

Mice were presented with two 1-minute feeding opportunities per session for six sessions with one such session given a day. The mice were divided into four groups, with two receiving a 10-minute interval between feeding opportunities and the other two a 60-minute interval. In each session mice were placed into the chamber with a 5-minute period before the first feeding opportunity was presented. Following this, the

interval of 10-minutes or 60-minutes was presented, occurring from the end of the first feeding opportunity to the start of the second, after which the second 1-minute long feeding opportunity was presented. At the end of this second opportunity the mice in both interval conditions were removed from the chamber. The running order of the four groups was intermixed so that across sessions the time of day was equated for both the 10-minute and 60-minute interval groups.

Results & Discussion

The data were split into two 1-minute time bins, each corresponding to the two 1-minute feeding opportunities and averaged over the six sessions. A mixed model ANOVA of 1-minute feeding opportunity x feeding opportunity interval was carried out to investigate the role of interval between feeding opportunities on palatability and intake.

Total Licks. The total number of licks made during consumption in the two 1-minute feeding opportunities before and after the 10-minute and 60-minute intervals are shown in the upper panel of figure 5. The 10-minute interval shows a slight increase in the second 1-minute feeding opportunity, whereas the 60-minute interval shows a similar number of licks before and after. The ANOVA showed that there was a significant main effect of 1-minute feeding opportunity F (1,22) = 17, $p < .001$, which also interacted with feeding opportunity interval F (1,22) = 6.9, p = .015. There was no significant main effect of feeding opportunity interval F (1,22) = .28, p = .59. Simple main effect analysis of the interaction found that there were no significant differences in the total number of licks across the first feeding opportunities F (1,22) = .04, p = .83 or the second F (1,22) = 2.05, p = .16. The 10-minute interval group did however have

a significantly higher total number of licks in the second feeding opportunity compared to the first F (1,22) = 22, $p < .001$, with no such significant difference in the 60-minute interval group F (1,22) = 1.1, p = .303.

Lick cluster size. The mean lick cluster size made during consumption in the two 1-minute feeding opportunities before and after the 10-minute and 60-minute intervals is shown in the lower panel of Figure 5. The 10-minute interval has a similar mean lick cluster size before and after, whereas after 60-minutes there is a decrease in the mean lick cluster size during the second feeding opportunity. The ANOVA showed that there was a significant main effect of feeding opportunity F (1,22) = 5.6, p = .026 and this interacted with feeding opportunity interval, F (1,22) = 6.7, p = .017. There was no significant main effect of feeding opportunity interval F (1,22) = 1.3, p = .25. Simple main effects analysis of the interaction further showed that there was no significant difference in the first feeding opportunity between the 5-second and 60-minute interval groups F (1,22) = .02, p = .87. There was however a significant difference in mean lick cluster size during the second feeding opportunity between groups F (1,22) = 4.4, p = .046, meaning that the mean lick cluster size was significantly lower after the 60-minute interval compared to a 10-minute interval group. The mean lick cluster size was also significantly lower during the second feeding opportunity of the 60-minute interval compared to the first feeding opportunity F (1,22) = 12, p = .002 but there was no significant difference between the 10-minute feeding opportunities F (1.22) = .02, p = .88.

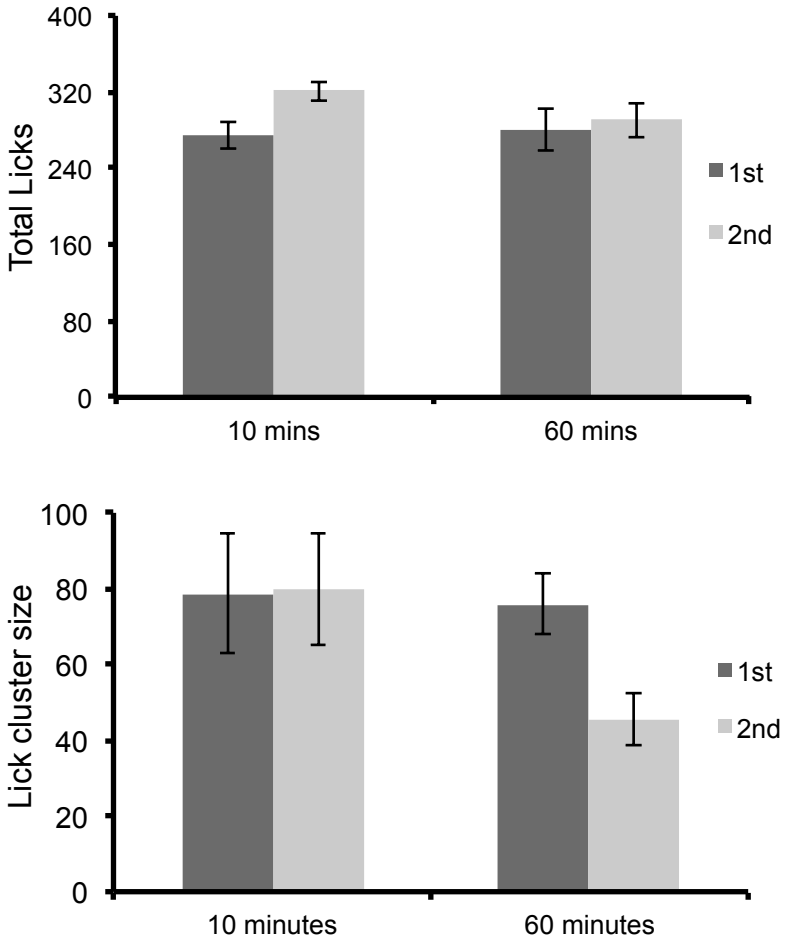

Figure 5. The total number of licks (top) and mean lick cluster size (bottom) for the 10 and 60-minute intervals during the two feeding opportunities. Error bars show ± standard error of the mean

There was a decline in the mean lick cluster size, but not the average total number of licks after a 60-minute interval between feeding opportunities, which is not seen after a 10-minute interval. This replicates the previous finding seen in experiment three, that a 60-minute interval results in a reduction in the palatability of the 16% sucrose solution when it is consumed during the second feeding opportunity. Furthermore, this decline is seen despite the use of a between-subjects design, meaning that the reduction in the perceived sweetness of the solution is unlikely to be due to the occurrence of frustrative non-reward, as there should be no expectation of a second feeding opportunity occurring before it is presented.

The lack of a reduction after a 10-minute interval also replicates the previous findings that this interval is sufficient for the recovery of the short-term adaptation effect that occurs with recent consumption. This further supports the idea that the decline in palatability after a 60-minute interval is due to a secondary adaptation effect, which occurs slightly later after consumption than the first rapid short-term adaption effect. Furthermore although this first adaptation effect reduces both the palatability and the total number of licks made during consumption in the second feeding opportunity, this second adaptation effect reduces only the palatability of the sucrose solution.

3.2: Experiment 5

Another confound which may have resulted in the second decline in palatability after a 60-minute feeding opportunity interval, is the increased time spent in the chamber during the 60-minute sessions. In the previous experiments when the second decline in palatability was seen, time spent in the context was not equated for each of the feeding opportunity intervals. As a result the 60-minute interval resulted in a greater length of time being spent in the chamber compared to either a 5-second or 10-minute interval. This increased time may result in factors such as stress reducing the palatability of the sucrose solution when it subsequently presented. The occurrence of stress has been proposed to alter regulation of eating behaviour (Woods & Begg, 2015) and has also been found to reduce the preference for a sweet saccharin solution (Harkin, Houlihan & Kelly, 2002). The aim of this experiment was therefore to test if time in the chamber results in the second decline in palatability, by equating this for all three of the feeding opportunity intervals. If the reduction in palatability after a 60-minute interval remains then this would provide further evidence for a second short-term adaptation process and not differences in time spent in the chamber.

In order to investigate this, all three of the feeding opportunity intervals were presented within each session to all animals. The feeding opportunities were 1-minute long with ten such opportunities presented during each session. This included one baseline presentation and three of each of the intervals, 5-seconds, 10-minutes and 60-minutes. In order to equate the previous time spent in the context, each of the different intervals were presented in three blocks of three, meaning that the third feeding opportunity in a block would always occur at the same time point during each

session. For example if one mouse received 5-seconds, 60-minutes then 10-minutes, another could have been presented with 60-minutes, 10-minutes and then 5-seconds. For each of these mice however, the third feeding opportunity occurs at the same time relative to the start of the session.

There are therefore three consistent time points across sessions in which a feeding opportunity occurs, allowing a comparison of the palatability after each of the different intervals independent of time previously spent in the context. Over three such sessions each animal receives three feeding opportunities following each of the intervals at a time equated across sessions, one of each interval during each session. These time matched feeding opportunities allow the reduction in palatability to be compared after each of the 5-second, 10-minute and 60-minute intervals between feeding opportunities. If there is a decline after 60-minutes independent of time spent in the chamber, then across sessions for each of the time matched points there should be a reduction seen after the three points in which there was a 60-minute interval between the current feeding opportunity and the previous. There should also be a reduction in palatability after a 5-second interval due to the more rapid short-term adaptation effect occurring after recent consumption, which has previously been shown to recover after a 10-minute interval.

Methods

Subjects & Apparatus

Forty-eight female C57 mice, bred in the life science support unit at Durham University were used. They were approximately between 5 and 8 months old at the start of testing and weighed between 16.8g and 26.3g. Mice were caged in groups of 4 to 12 in a temperature controlled housing room with a 12hr light-dark cycle. During testing they were maintained at 85% of their free feeding body weights with ad libitum access to water in their home cages. Apparatus was the same as that used in the previous experiment.

Procedure

Mice were given three sessions in which they were presented with ten feeding opportunities to consume 16% sucrose solution, each of which was 1-minute long, with one such session a day. The first baseline feeding opportunity occurred when the sipper tube was presented after 5-minutes of time spent in the chamber. The subsequent nine 1-minute long feeding opportunities were each presented following one of the three different intervals, 5-seconds, 10-minutes or 60-minutes, from the previous feeding opportunity ending to the next one starting by the sipper-tube being reinserted into the chamber. These were presented in an intermixed order in blocks of three, with one interval of each duration occurring in every three feeding opportunity presentations. As a result the baseline, fourth, seventh and tenth feeding opportunities all occurred at the same time relative to the start of the session, across sessions. The order of the intervals presented in each of the blocks of three was

intermixed so that in each session one of the three intervals occurred at one of the three matched time points. These orders were also intermixed across sessions, so that each animal received all of the three intervals at each of the three different time matched points across the three testing sessions. Table 1 shows this intermixed order of feeding opportunity intervals presented to the first eight mice during the three sessions, including baseline and the following nine presentations. Each of the

subsequent five groups of eight mice received a different unique order of presentations so that the ordering was counterbalanced within and across sessions.

Table 1. The order of presentations for the 5-second, 10-minute and 60-minute feeding opportunity intervals during the first three sessions. The time matched points are shown in grey.

	Interval orders									
1	B	5	10	60	10	60	5	60	5	10
2	B	10	60	5	60	5	10	5	10	60
3	B	60	5	10	5	10	60	10	60	5

Results & Discussion

The three time-matched feeding opportunities for each feeding opportunity interval were averaged to get a mean lick cluster size and average total number of licks for each of the three intervals. A repeated measures ANOVA of feeding opportunity interval was then carried out on the time matched data.

Total Licks. The average total number of licks made during baseline and after the three feeding opportunity intervals is shown in the top panel of Figure 6. This shows a decline after a 5-second interval compared to a 10-minute interval and a slight decline after a 60-minute interval, with baseline similar to a 10-minute interval. The ANOVA, not including baseline, showed that there was a significant main effect of interval between feeding opportunities on the total number of licks made during consumption F (2,94) = 33.3, $p < .001$. Post hoc analysis using the bonferroni correction for multiple comparisons revealed that the total number of licks after a 5-second interval was significantly lower than after a 10-minute interval ($p < .001$) as well as a 60-minute interval ($p < .001$). The number of licks after a 10-minute interval approached being significantly higher than after a 60-minute interval ($p =. 072$).

Lick cluster size. The mean lick cluster size during consumption after each of the three feeding opportunity intervals is shown in the lower panel of figure 6, with the 5-second as well as the 60-minute intervals both showing a reduction in palatability compared to the 10-minute interval and baseline. The ANOVA revealed that there was a significant main effect of feeding opportunity interval on the mean lick cluster size during consumption, $F (2,94) = 14$, $p < .001$. Post hoc analysis using the bonferroni correction for multiple comparisons further showed the mean lick cluster size is significantly lower after a 5-second interval than after 10-minutes ($p < .001$) as well as 60-minutes ($p = .008$). The mean lick cluster size after a 10-minute interval is also significantly greater than after a 60-minute interval ($p = .025$).

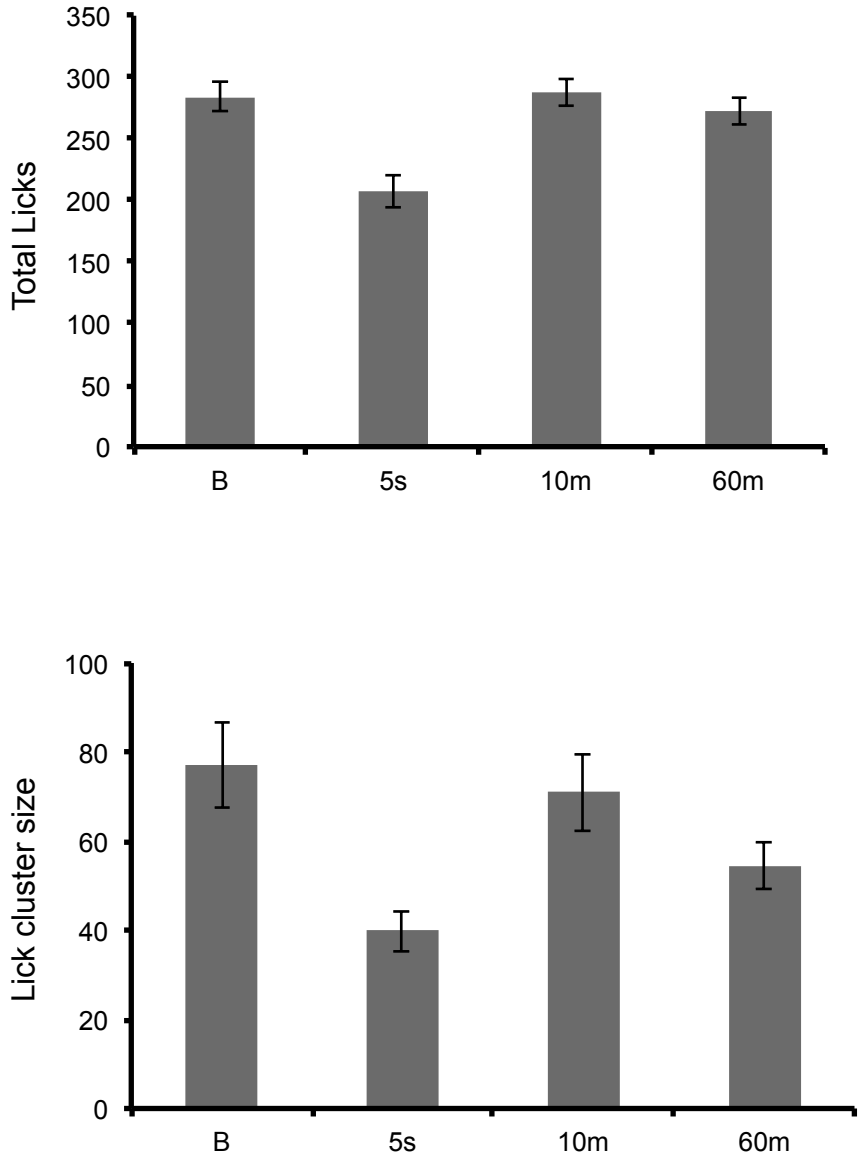

Figure 6. The total number of licks (top) and mean lick cluster size (bottom) during consumption of the sucrose solution at baseline and after the three different feeding opportunity intervals. Error bars show ± standard error of the mean

The decline in palatability after a 60-minute interval remains despite differing start times of the feeding opportunities and resulting time in the context being equated. This means that any influence of additional time in the box, such as increased stress, is not the cause of the second reduction in palatability. Therefore this experiment provides further evidence for a second short-term adaptation effect, which again appears selective for a reduction in mean lick cluster size and palatability.

As well as controlling for time in the context, this experiment also differed from the previous experiments in that it involved the presentation of more than two feeding opportunities in each session. As a result of this, each of the time matched feeding opportunities occurring in each session are presented following at least two other feeding opportunity intervals. This demonstrates that the inverted U-shape function of palatability remains even when multiple intervals and feeding opportunities occur in succession. Importantly this means that the mean lick cluster size remains greater after a 10-minute interval, even when on some occasions this follows the reduction seen after a 60-minute interval. Therefore the reduced mean lick cluster size that occurs after a 60-minute interval can recover quickly when another feeding opportunity is presented. Furthermore palatability continues to be reduced after a 60-minute interval, even when shorter intervals have occurred previously within the same session. This finding has implications when considering the potential mechanisms behind the secondary decline in palatability, as rather than there being a secondary slow acting process that takes time to recover, it can instead recover with further consumption of sucrose shortly after the 60-minute interval has occurred.

3.3: Discussion

The experiments in this chapter investigated two potential factors contributing to the second decline in palatability seen after a 60-minute interval between feeding opportunities. One potential explanation for this reduction was the previous use of a within-subjects design, which may have resulted in the second feeding opportunity being expected before the 60-minute interval. This may have resulted in the occurrence of frustrative non-reward when the sucrose solution fails to be presented earlier in the session, reducing the palatability of the sucrose solution when presented after 60-minutes. Experiment four investigated the role of a within-subjects design by replicating the same 10-minute and 60-minute intervals between feeding opportunities using a between-subjects design. Despite the 60-minute group having no previous experience of any shorter interval between feeding opportunities, there remained a decline in palatability, but not total licks, in the second feeding opportunity. There is therefore a secondary decline in palatability of the sucrose solution that is not due to the within-subjects design previously used in experiment three.

Another potential factor contributing to this decline in palatability, is that the presentation of the second feeding opportunity occurred at different times relative to the start of the session for each of the three intervals. The increased time spent in the chamber before presentation of the sucrose solution, may have resulted in a decline in palatability independent of the time since last consumption. During the 60-minute interval the influence of this factor would be greatest, with a 10-minute interval potentially not being a sufficient duration for time in the context to greatly alter

palatability. Experiment five therefore equated the time at which the second feeding opportunity was presented, by providing three time matched opportunities in each session. This allowed for the palatability and the total number of licks made during the second feeding opportunity to be compared after each of the three different intervals. As with the previous experiments, there remained a reduction in palatability after both a 5-second and a 60-minute interval between feeding opportunities, with no such reduction in either measure after a 10-minute interval. There is therefore a secondary decline in palatability, but not the total number of licks made during consumption of 16% sucrose solution, which is not due to either the previous use of a within subjects design or confounded start time of the second feeding opportunity.

Chapter Four

Discussion

4.1: Summary

The aim of this thesis was to investigate the short-term adaptation that occurs after recent consumption, with this adaptation seen in the amount consumed reducing as well as the palatability of the food being reduced. In particular the time course of this adaptation to sucrose solution in mice was investigated, with mean lick cluster size as a measure of palatability. GluA1 knockout mice were also tested to investigate the role of the GluA1 subunit of the AMPA receptor in the short-term adaptation that occurs after recent consumption.

The first experiment demonstrated that short-term adaptation occurs in mice after recent consumption of sucrose solution, resulting in reduced intake and palatability. This reduction in the mean lick cluster size is similar to that seen when the concentration of the sucrose solution is reduced, with lick cluster size showing a monotonic increase with concentration (Austen, Strickland & Sanderson, 2016; Dwyer, 2008; Spector, Klump & Kaplan, 1998), suggesting that the perceived sweetness of the sucrose reduces with recent consumption. Experiment two found that the reduction in palatability was greatest during a more massed schedule, meaning that it can recover to some extent over short 4-minute intervals. Experiment three then further investigated the time course of the adaptation effect, by spacing two feeding opportunities by three different intervals, of 5-seconds, 10-minutes and 60-minutes. It was found that rather there being only a single adaptation process that recovers over increasing intervals, there was an inverted U-shape function of palatability with time

since recent consumption. This experiment also found that although GluA1 deletion does result in a general reduction in intake and palatability, the short-term adaptation effects are not GluA1 dependent.

Experiments four and five then ruled out two potential experimental confounds which may have resulted in the second reduction in palatability after a 60-minute interval. Firstly, the role of conditioned frustration that may have resulted from the previous use of a within-subjects design was tested, by replicating the experiment using a between-subjects design. Secondly any effects of increased time spent in the chamber, such as stress, were also investigated by equating the time in the context for all three intervals. In both of these experiments the reduction in mean lick cluster size remained after a 60-minute interval, providing further evidence for a second short-term adaptation process. Experiment five further found that palatability remains higher after a 10-minute interval, even when this has, on some occasions, occurred after a 60-minute interval.

There therefore appears to be an inverted U-shape function on palatability that occurs with time since recent consumption, resulting in a reduced mean lick cluster size after both a 5-second and 60-minute interval between feeding opportunities. This suggests that there are two processes of short-term adaptation occurring after recent consumption of a sucrose solution. The first acts rapidly to reduce the total number of licks and mean lick cluster size during consumption, with recovery occurring during a 10-minute interval. In contrast the second process appears selective for a reduction in palatability, seen only significantly in the mean lick cluster size and not the total number of licks. This second process also has a longer time course, with the influence

seen after a 60-minute interval and not after a 10-minute interval between feeding opportunities. Furthermore this continues to occur even when multiple feeding opportunities and intervals are presented in each session.

4.2: The first short-term adaptation effect

This finding of a rapid short-term adaptation effect after recent consumption, which results in a reduction of both amount consumed and the palatability, is in line with previous studies demonstrating such an adaptation effect in both human and animal studies (e.g. Berridge, 1991; Hetherington, Rolls & Burley, 1989). There are however a number of potential explanations for this reduction in responding seen after recent consumption, including fatigue, sensory adaptation and short-term memory processes.

Fatigue could explain the reduction in both the total number of licks and the volume consumed, as being due to a general reduction in responding that occurs with consumption. Such an explanation can be ruled out by demonstrating the decline in responding to be specific to the food consumed. In these experiments as only a single sucrose solution and flavour was presented throughout, it cannot be concluded that the reduction in intake and palatability is not due to a general reduction mechanism. Other similar experiments in rodents however, have demonstrated that the reduction in responding is sensory-specific. For example Berridge (1991) using taste reactivity to measure palatability found sensory-specific satiety to solutions of sucrose or milk, when the same solution had previously been consumed. Furthermore Rolls, Duijvenvoorde & Rowe (1983) found increased consumption in rats when a variety of foods were given in succession as opposed to the same food being repeatedly presented, suggesting there was a reduction in the short-term adaptation effect when a variety of foods is presented. Similarly, sensory adaptation could also explain the adaptation effect as due to a reduction in responsiveness to the sucrose solution being consumed. This would result in a corresponding decline in the perceived intensity of

the stimulus, which in the case of the sucrose solution may reduce the perceived sweetness and palatability of the solution to also reduce consumption. Ruling out such sensory adaptation in animal studies is difficult, although in human studies it has been observed that intensity ratings do not correlate with reduction in palatability and consumption (Rolls, Rolls & Rowe, 1983). Although it remains unclear as to the role of sensory adaptation, that may occur after recent consumption in these studies using licking analysis in mice.

Another potential mechanism resulting in the reduction is short-term memory, with the stimulus representation reducing responding after recent presentation (e.g. Wagner, 1981). Deletion of the GluA1 subunit of the AMPA receptor results in a failure to reduce responding to recently experienced spatial (Sanderson et al, 2009) as well as non-spatial (Sanderson et al, 2011) stimuli. This subunit of the AMPA receptor has therefore been related to the expression of short-term memory and the reduction in responding normally seen after recent stimulus presentation (Sanderson et al, 2010; Sanderson, Sprengel, Seeburg, & Bannerman, 2011). In experiment three however it was found that although the GluA1 knockout mice did show a general reduction in mean lick cluster size, as well as the total number of licks, the same inverted U-shape function of palatability remained. Therefore, the short-term adaptation effect seen after recent consumption of sucrose solution does not depend on the GluA1 subunit of the AMPA receptor. This contrasts to the previous findings in which GluA1 deletion does result in a failure to reduce responding, suggesting that the reduction after consumption of sucrose may relate to a different mechanism than the GluA1 dependent habituation occurring after other stimulus presentations.

As well as the GluA1 subunit being associated with reduced responding after stimulus presentation, there is also a range of evidence suggesting that sensory processes are important in adaptation. For example Berridge (1991) found that short-term adaptation in rats after recent consumption was sensory-specific to the solution consumed. Further evidence for the role of sensory processes, comes from the reduced intake of glucose after recent consumption of saccharin (Hsiao & Tuntland, 1971; Jones, 1971). This demonstrates that it is the sensory experience, rather than any negative feedback mechanisms resulting in the adaptation effect that occurs after recent consumption. As a result the decline seen in these experiments after a short 5-second interval, likely also reflects sensory-specific satiety (e.g. Rolls, 1986). However in order to fully conclude sensory processes relate to the short-term adaptation seen in this series of experiments, these would have to be repeated with a second flavour also used. If the reduction in total licks and mean lick cluster size occurs only to the previously experienced flavour, it can be concluded to reflect sensory-specific satiety mechanisms.

4.3: The second decline in palatability

The second decline in palatability that is seen after a 60-minute interval appears to reflect a different short-term adaptation process, due to recovery of the first adaptation process after a 10-minute interval. As with the initial adaptation effect there are various mechanisms that may result in this secondary adaptation effect, including negative feedback mechanisms and memory processes. Two factors that are unlikely to be related to this decline in palatability are fatigue and sensory adaptation, due to recovery of the total number of licks and the mean lick cluster size occurring after a shorter 10-minute interval. Furthermore the GluA1 knockout mice show the second decline in palatability and this subunit being associated with reduced responding and short-term memory (Sanderson, Sprengel, Seeburg & Bannerman, 2011), suggesting this second reduction is independent of these mechanisms.

Another factor that may influence this secondary adaptation effect is that of homeostatic processes and negative feedback signals, which act to regulate energy intake (Woods & Langhans, 2012; Woods & Begg, 2015). The influence of these negative feedback signals necessarily relies on the ingestion of the food having occurred, with Booth et al (1970) suggesting that any post-absorptive effects should not influence eating behaviour until fifteen minutes after consumption. Similarly Cabanac (1971) also suggested a slow time course of adaptation after consumption, occurring from twenty minutes onwards with a maximal decline at around an hour. Before this time, any changes in consumption must relate only to the sensory properties of the food being consumed, due to sufficient absorption not yet taken place. This further supports the initial adaptation effect after a 5-second interval being

related to sensory mechanisms and suggests the second decline in palatability after a 60-minute interval, may relate to negative feedback mechanisms. However, as the studies by Cabanac (1971) and Booth (1970) relate to the time course of digestion in humans, the time course of digestion in mice remains unclear. Furthermore, a single minute of access to the sucrose solution may not result in sufficient consumption for negative feedback mechanisms to reduce palatability an hour after intake.

To understand the potential role of negative feedback after consumption, the process of digestion and resulting feedback signals in mice needs to be considered. In rodents this process begins with taste information being conveyed to the nucleus of the solitary tract (NTS) with projections from this to the medial parabrachial nucleus (PBN) (Rolls, 2015). The PBN appears to have an important role in directing eating behaviour, with diverging parallel ventral and dorsal projections relaying hedonic and sensory properties respectively (Rolls, 2015; Scott & Small, 2009). In both the NTS and the PBN, taste evoked activity has been found to be function of concentration of the solution being consumed, including sucrose concentration (Giza & Scott, 1987a; Scott & Small, 2009). A reduction in activity in the NTS and the PBN therefore relates to a reduction in the perceived concentration or intensity of the solution. Furthermore responding has also been found to relate to the physiological state of the animal, for example during sodium deprivation, which appears to alter the responding to salt solutions in a manner that increases the hedonic value (Jacobs, Mark & Scott, 1988). The NTS and PBN therefore appear important in both sensory and hedonic processing of taste stimuli, altering depending on the physiological state of the animal and acting to direct subsequent eating behaviour.

As well as sodium depletion, taste evoked activity in the NTS has also been found to be determined by the blood glucose concentration (Giza & Scott, 1983; Giza & Scott, 1987a). For example Giza and Scott (1987a) found that intravenous glucose injections resulted in a decrease in taste evoked activity and lick rates mirroring that of a reduction in actual glucose concentration. As well as glucose directly altering NTS taste evoked activity insulin infusions have also been found to have similar effects in reducing responsiveness (Giza & Scott, 1983), with insulin important in the regulation of blood glucose concentration. Therefore taste evoked activity in the NTS and the PBN, an area relating to the hedonic and sensory processing taste stimuli, may relate to the decline in responding seen during the adaptation that occurs after consumption of sucrose. Furthermore this taste evoked activity depends on post-absorptive effects, meaning they are unlikely to account for the immediate short-term adaptation effect.

However it remains difficult to conclude a role for such taste evoked responding relating to blood glucose concentration in the second decline in palatability seen in this series of experiments. Firstly, the time course in the experiments conducted by Giza and Scott (1987a, 1987b, 1983) were shorter than the 60-minutes used in this series of experiments, with reduced responsiveness occurring around twenty minutes after a glucose load or insulin infusion. This suggests that negative feedback signals occurring as a result of recent consumption in rodents occur before 60-minutes, making the role of these in the inverted U-shape function of palatability seem less likely. It may be the case however, that there is a slow recovery of the decline in responsiveness in the NTS and PBN, meaning such processes are able to explain the reduction seen after 60-minutes. However the finding from experiment five, in which palatability remains

higher after a 10-minute interval even when this sometimes occurs after a 60-minute interval, questions the role of a slow acting homeostatic process. More specifically if there is a slow acting process with a slow recovery period, then there should be no recovery when a subsequent 10-minute interval is given following a 60-minute interval. Furthermore it remains unclear how such negative feedback mechanisms, which have an important role in the regulation of energy intake and homeostasis, would only result in a reduction in palatability and not consumption. If negative feedback does result in the secondary decline, then it would be expected that not only would the palatability be reduced but also the amount consumed, as this would ensure consumption and energy intake is regulated. Although it could be the case that greater consumption of sucrose would lead to a reduction in both palatability and intake, with only a weak feedback effect seen in the current series of experiments.

Although there may be a role for negative feedback mechanisms, another explanation for the second decline in palatability is that it relates to memory processes. Despite these two reductions in palatability being GluA1 independent, memory may still be underlying the inverted U-shape function of palatability seen after recent consumption. In particular one model that could account for the two reductions in palatability is AESOP, the affective extension of SOP (Wagner & Brandon, 1989). This proposes that the representation of the unconditioned stimulus, in this case the sucrose solution, consists of both a sensory and an affective component or 'node'. The sensory node corresponds to the specific sensory qualities of the stimulus, whereas the affective node relates to the emotive properties associated with the stimulus being presented. Both of these nodes are activated upon presentation of the unconditioned

stimulus and enter the primary active state (A1), before decaying to a secondary active state (A2) and finally to an inactive state of memory. However the two different representative nodes decay at different rates from the A1 state, with the sensory node decaying more rapidly between states than the affective node. As a result of this, the sensory and affective components of the stimulus representation may be in different states of memory at any one time, with responding reducing as a node decays from the A1 state to the A2 state of memory.

The differing time courses of the two representative nodes may therefore explain the two reductions in palatability seen in this series of experiments. In particular, the sensory representation of the sweet sucrose solution will decay more rapidly to the A2 state, reducing the palatability of the sucrose rapidly after consumption. In contrast the affective representation of the sucrose, which may contain the positive emotional response that occurs after consumption, will decay into the A2 state at a slower rate and also remain in this secondary active state for longer. As a result the reduction in responding that occurs as a representation decays into the A2 state, will also occur over a longer time course for the affective representational node. This could explain the reduction in palatability after a 60-minute interval as being due to the positive affective node being in the A2 state and reducing palatability when consumption of the sucrose occurs. One way to test if the second decline in palatability relates to the affective properties of the sucrose would be to use non-calorific sweetener, as this may remove the positive emotional response resulting from recent consumption of sucrose. If the second decline in palatability remains even with the use of sweetener,

then this would suggest that the reduction in responding is not due to the affective representation decaying to the A2 state of memory.

4.4: Final conclusions

The overall aim of this thesis was to investigate the short-term adaptation effect that occurs after recent consumption of sucrose solution in mice. It has been shown that rather than a single short-term adaption acting to alter eating behaviour after consumption, there is an inverted U-shape function of palatability with time since consumption of sucrose solution. This suggests there are two short-term adaptation processes that occur after recent consumption of sucrose, both of which act to reduce the palatability of the solution during subsequent consumption. The first short-term adaptation effect may reflect sensory-specific mechanisms, acting to rapidly reduce intake as well as the palatability of the food being consumed. However, the mechanism behind the second decline in palatability 60 minutes after consumption remains unclear. This finding that there is not just one short-term adaptation process after recent consumption, has important implications for eating behaviour and its disorders. In particular, research into eating behaviour needs to consider the influence of multiple short-term adaptation processes with differing time courses that occur after recent consumption. These processes may act together to control energy intake not only over shorter durations within a meal, but also over longer periods to regulate intake across meals. Importantly when investigating the altered regulation of such intake, such as during over and under eating, the role of each of these different adaptation processes need to be considered.

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